

ABILITY OF CARIES DETECTION METHODS
TO DETERMINE CARIES LESION
ACTIVITY

by

Fatma Aldawood

Submitted to the Graduate Faculty of the School of
Dentistry in partial fulfillment of the requirements
for the degree of Master of Science in Dentistry,
Indiana University School of Dentistry, December
2019.

Thesis accepted by the faculty of the Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

Kim E. Diefenderfer

Anderson T. Hara

Masatoshi Ando
Chair of the Research Committee

Date_____

DEDICATION

I dedicate this work to my beloved parents
for their guidance, support and faith in me.

I owe it all to you.

ACKNOWLEDGMENTS

First, I would like to express my gratitude to Allah for his countless blessings, for giving me the strength, the opportunity and the drive I needed to accomplish my goals.

Second, I would like to thank my parents for their infinite love and support, without which I would not have gone this far.

I would like to thank my mentor, Dr. Masatoshi Ando, for his incredible efforts, his guidance and his patience, and for providing me with enlightenment and feedback throughout this project.

I would like to thank the members of my committee, Drs. Kim Diefenderfer, Anderson Hara, and Norman B. Cook, my program director, for their support.

Special thanks to Drs. Diefenderfer, Armando Soto and Haixia Xing for their cooperation, and for taking the time to participate in this project. I'm deeply grateful for the efforts they made to make this project happen.

Finally, I would like to thank Delta Dental Foundation for providing the project with financial support. I would like to express my appreciation to my sponsors, SACM and Armed Forces Medical Services of Saudi Arabia, for granting me this opportunity to pursue my goals.

TABLE OF CONTENTS

Introduction.....	1
Caries prevalence	2
White spot lesions/non-cavitated early lesions	3
Caries lesion severity and activity	4
Surface layer thickness	5
Gaps of knowledge	7
Specific aims	9
Hypotheses	10
Review of Literature	11
Visual and tactile examination/International Caries Detection and Assessment System (ICDAS).....	12
Microcomputed tomography validity as a gold standard method.....	14
Quantitative light-induced fluorescence	17
Materials and Methods.....	21
Sample selection and preparation	22
Visual and tactile examination.....	23
Training.....	24
Specimen selection.....	24
Surface optical profilometry	25
Microfocus computed tomography (μ CT) imaging.....	26
Quantitative light-induced fluorescence imaging (QLF) during dehydration	27
Statistical analyses	28
Sample size justification	28
Results.....	29
Visual and tactile examination.....	30
Surface roughness (RA) (μ m).....	30
Surface layer thickness	31
Gray values:	31
Lesion depth (μ m).....	31
Lesion volume (mm^3)	32
QLF ΔQ at 15 seconds of dehydration (ΔQ_{15})	32
QLF of dehydration (ΔQ_D).....	32
Associations of ΔQ_D with surface roughness.....	33
Associations of ΔQ_D with micro-CT surface layer thickness	33
Associations of ΔQ with micro-CT lesion depth and lesion volume.....	33
Associations of surface roughness with micro-ct surface layer thickness	34

Tables and Figures	35
Discussion	78
Summary and Conclusions	89
References	93
Abstract	100
Curriculum Vitae	

LIST OF ILLUSTRATIONS

TABLE I	ICDAS criteria for the study teeth selection	36
TABLE II	ICDAS caries lesion activity assessment	37
TABLE III	Analysis of visual and tactile evaluations to determine caries activity.....	38
TABLE IV	Comparisons between teeth with and without visual indications of caries activity and surface roughness (RA) (μm).....	39
TABLE V	Comparisons between two-sample t-test, mean (\pm sd) surface layer thickness (mm) of teeth with and without visual indications of caries activity	40
TABLE VI	Two-sample t-test, comparisons between teeth with and without visual indications of caries activity and gray values for sound tissue.....	41
TABLE VII	Two-sample t-test, comparisons between teeth with and without visual indications of caries activity and lesion depth (μm)	42
TABLE VIII	Two-sample t-test, mean (\pm sd) lesion volume (mm^3) in teeth with and without visual indications of caries activity.....	43
TABLE IX	Two-sample t-test, mean (\pm sd) change in fluorescence (ΔQ) after 15 seconds dehydration in teeth with and without visual indications of caries activity and QLF ΔQ at 15 seconds dehydration.	44
TABLE X	Two-sample t-test, mean (\pm sd) change in fluorescence [ΔQD ($\% \times \text{mm}^2/\text{sec}$)] (QLF 5% threshold) after varying dehydration times in teeth with and without visual indications of caries activity.....	45
TABLE XI	Pearson correlation coefficients of associations of QLF ΔQD ($\% \times \text{mm}^2/\text{sec}$) with surface roughness (RA) (μm)	46
TABLE XII	Pearson correlation coefficients of associations of QLF ΔQD ($\% \times \text{mm}^2/\text{sec}$) with μCT surface layer thickness	48
TABLE XIII	Pearson correlation coefficients of associations of QLF ΔQ with μCT lesion depth (15 seconds dehydration).....	50
TABLE XIV	Pearson correlation coefficients of associations of QLF ΔQ with μCT lesion volume	51

TABLE XV	Pearson correlation coefficients of associations of surface roughness with μ CT surface layer thickness.....	52
FIGURE 1	Lesion volume (mm^3) in teeth with and without visual indications of caries activity.....	53
FIGURE 2	Lesion depth (μm) in teeth with and without visual indications.....	54
FIGURE 3	Comparisons between teeth with and without visual indications of caries activity and QLF ΔQ at 15 seconds of drying.....	55
FIGURE 4	Associations of QLF ΔQ at 15 sec of dehydration with μ CT lesion depth and lesion volume (Pearson correlation coefficient).....	56
FIGURE 5	Comparisons between teeth with and without visual indications of caries activity and surface layer thickness (mm).....	57
FIGURE 6	Comparisons between teeth with and without visual indications of caries activity and surface roughness (R_a) (μm).....	58
FIGURE 7	Comparisons between teeth with and without visual indications and surface roughness (R_a), (R_a lesion - R_a sound / R_a sound).....	59
FIGURE 8	Comparisons between teeth with and without visual indications of caries activity and ΔQD ($\% \times \text{mm}^2/\text{sec}$).....	60
FIGURE 9	Scatterplot of correlation of QLF ΔQD ($\% \times \text{mm}^2/\text{sec}$).....	61
FIGURE 10	Scatterplot of correlation of QLF ΔQD ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-min.....	62
FIGURE 11	Scatterplot of association of QLF ΔQ with μ CT lesion depth (μm)	63
FIGURE 12	Scatterplot of association of QLF ΔQ with μ CT lesion volume (mm^3) significantly correlated, with a moderate positive association.....	64

FIGURE 13	Sample preparation on Lego® bricks.....	65
FIGURE 14	Metal plates used for training with a 2×5mm window opening and a scale to measure the amount of force exerted while probing.....	66
FIGURE 15	Tactile examination using a #6 Handle-Satin Steel explorer (23 SE, Hu-Friedy Mfg. Co., Inc., USA).....	67
FIGURE 16	QLF during dehydration assembly.....	68
FIGURE 17	QLF image analysis.....	69
FIGURE 18	Micro-CT imaging and analysis.....	70
FIGURE 19	The region of interest function in CTAn software was used to isolate the surface layer in enamel lesions.....	71
FIGURE 20	The region of interest function in CTAn software was used to isolate the enamel lesions.....	72
FIGURE 21	The region of interest function in CTAn software was used to isolate the dentin lesions.....	73
FIGURE 22	3D reconstruction of surface layer of non-cavitated white spot enamel lesion from μCT image analysis.....	74
FIGURE 23	3D reconstruction of enamel lesion in non-cavitated white spot enamel lesion from μCT image analysis.....	75
FIGURE 24	3D reconstruction of dentine lesion in non-cavitated white spot enamel lesion from μCT image analysis.....	76
FIGURE 25	Non-contact profilometric analysis.....	77

INTRODUCTION

CARIES PREVALENCE

Dental caries imposes a burden worldwide and is considered the most widespread non-communicable disease.¹ According to the American Academy of Pediatric Dentistry,² caries in children is five times more prevalent than asthma. Forty-five percent of children ages 5 to 17 have caries, and the problem is particularly severe among specific populations such as low-income children. Although a great decline in dental caries prevalence is noted in high-to-middle-income countries, the burden of dental caries is increasing.³ The increased global burden of dental caries is mainly attributed to an amplified number of retained teeth throughout the world from 1990 to 2010, population growth and longevity.³

Even though evidence is limited from systematic reviews of the epidemiology of dental caries worldwide, the prevalence is great.⁴ Untreated dental caries in permanent teeth was the most prevalent condition reported globally in 2010.⁵ Untreated dental caries presents a biological, social and financial burden on individuals and healthcare systems.⁵ According to the World Health Organization (WHO), caries is considered the fourth most expensive chronic disease to treat, with a projected spending of \$122 billion in the US alone in 2014 (Centers for Medicare and Medicaid Services 2011).⁵ If left untreated, dental caries can cause pain and infection degrading the quality of life for both adults and children, who manifest lower productivity, poorer school attendance and decreased overall performance.⁵

The role of dental practitioners is to help patients to control their caries disease process. Early detection enables dentists to assess caries lesions before they progress to cause cavitation, pain, and eventually tooth loss, which could force the patient to seek urgent treatment for impairment of masticatory function, speech, and esthetics.⁶ Therefore, early detection of non-cavitated lesions is essential to shift treatment decisions towards preventive (non-invasive) rather than restorative (surgical) modalities.

WHITE SPOT LESIONS/NON-CAVITATED EARLY LESIONS

White spot lesions or non-cavitated early lesions represent the initial clinical observation of caries. They occur due to acid diffusion through the enamel surface, demineralizing the enamel subsurface, but leaving the surface layer intact.⁷ If demineralization continues, then the progression of the lesion eventually causes the enamel surface to collapse, creating cavitation.^{7,8} Clinically, these white spot lesions lose the translucency of normal enamel due to altered light properties, resulting in a white “chalky” appearance, especially when dehydrated.⁹ The surface layer is fragile and easily damaged, and the increase in porosity of the subsurface renders it susceptible to staining. The reduction in density of the subsurface results in a change in the refractive index of the affected area, with increased surface roughness, loss of luster, and alteration in internal reflection. The overall result is an increased visual opacity of the enamel,¹⁰ which may be detectable by radiography, transillumination, and laser-detecting devices.

CARIES LESION SEVERITY AND ACTIVITY

The terminology related to caries detection and diagnosis is often confusing in the literature. For this reason, it is essential to distinguish these terms. Pitts¹¹ suggested that “caries lesion detection” refers to methods by which the presence or absence of the disease is determined. As opposed to the conventional belief, caries is not a continuous process, but alternates between periods of activity and inactivity. When the outcome of these processes over time is a net loss of minerals, the lesion develops or progresses. On the other hand, if mineral deposition dominates, lesion progression is arrested.¹² Nyvad et al.¹² introduced clinical criteria for caries detection and activity assessment. Caries lesion activity is determined by surface texture and reflectivity. Rough, chalky lesions are considered to be active, while hard, smooth, and shiny lesions are characterized as inactive. Nyvad et al.¹³ verified this theory with a three-year longitudinal study, which showed high predictive ability of these caries detection and activity assessment criteria.

There is a consensus among recognized cariology experts that detection of caries lesions and determination of their severity are not enough to reach an accurate diagnosis.¹¹⁻¹⁶ Active non-cavitated lesions are at a 60-percent higher risk of progression to cavitation than inactive lesions.^{14,17} Determination of caries lesion activity is important to stop lesion progression. Incorrect diagnosis, whether a lesion is active or inactive/arrested, can easily result in improper treatment decisions, specifically regarding irreversible surgical treatments.

SURFACE LAYER THICKNESS

Kidd¹⁸ studied enamel caries lesions in teeth extracted from young patients for orthodontic purposes and lesions in teeth extracted from patients over 65 years for periodontal reasons. They pointed out that the main histologic differences between young and old lesions were in the prevalence and position of the dark zones. The high prevalence of laminations within the body of old lesions indicates temporary arrest of the caries lesion. The author concluded that active lesions present thin surface layers compared with older, arrested lesions. Ekstrand et al.¹⁹ noted that there is a strong correlation between histologic depth of the lesion and visual criteria in active lesions. They concluded that clinicians can detect caries lesions and predict their activity and severity based on visual and tactile examination, similar to previous findings.¹²

In 1979, Ten Bosch²⁰ implemented a non-destructive method using light scattering to obtain semi-quantitative information suitable for monitoring the demineralization and remineralization processes. The results of the study indicated that deeper lesions caused drastic change in light flux. In a later *in-situ* study, Arends et al.²¹ compared 10 different experimental techniques, including microradiography, polarized light microscopy, and light scattering, to assess the amount of enamel de- and remineralization. Transverse microradiography (TMR) was the most practical and most widely accepted direct technique, while light scattering and microhardness tests proved to be acceptable as indirect techniques. Jones and Fried²² indicated that the thickness of the surface layer/zone increased for the lesions exposed to the fluoride remineralization solution when tested by TMR. Neuhaus et al.¹⁴ employed optical reflectivity, using an experimental optical sensor, in the determination of caries. They found significantly

lower values in optical reflectivity of active lesions when compared with inactive lesions. Jaruszewski²³ confirmed with a scanning white-light interference microscope that the surfaces of active lesions had high porosity and more than ten-fold greater roughness than inactive lesions, which exhibited almost no porosity.

Several studies investigated the surface layer thickness (SLT) of enamel lesions, but none of them managed to assess differences in SLT between active and inactive early enamel lesions.²⁴⁻²⁸ An *in-vitro* study by Kielbassa et al.²⁴ indicated that the mean surface layer in artificial lesions of bovine enamel is 15 μm to 16 μm , measured by microradiography. This average was smaller compared with other studies. Meyer-Lueckel et al.²⁵ reported a mean of 30 μm to 40 μm in natural proximal enamel lesions using laser scanning microradiography for imaging, but 29 percent of the measured surface layers were over 50 μm . According to Cochrane et al.,²⁶ the thickness of the surface layer in natural proximal enamel lesions, measured by four methods of calculation, ranged from 35 μm to 130 μm . The majority was 35 μm to 60 μm with no significant difference between active and inactive lesions, although active and inactive lesions showed different levels of mineral content.²⁶ Active lesions had less mineralized and more porous surface layers than inactive ones.²⁶ Shahmoradi and Swain²⁷ used x-ray micro-computed tomography (μCT) imaging, a modified version of medical computed tomography imaging, which can attain higher resolution images and good analytical data to measure the surface layer of natural enamel lesions in fissures and proximal surfaces. They found that fissural lesions had a surface layer thickness ranging from 0 μm to 137 μm with a mean of 85 μm , while proximal lesions had a range of 0 μm to 90 μm with a mean of 56 μm . Another study compared the surface layer thickness of natural white spot

lesions (WSL) with brown spot lesions (BSL) using μ CT imaging.²⁸ BSL are presumed as arrested and partially remineralized, possibly attaining the brown stain through surface absorption of organic materials and minerals.²⁸ The study found that the SLT of WSL ranged from 0 μ m to 90 μ m, while BSL ranged from 20 μ m to 250 μ m, with averages of 51 μ m and 79 μ m respectively.²⁸

GAPS OF KNOWLEDGE

There is no consensus on definitive indicators to assess caries activity, and the current clinical assessments are a “guess-estimate” at best. The most commonly used methods of lesion assessment are comprised of visual and tactile examinations, which are prone to subjective bias. Furthermore, they are limited to exposed surfaces, and potential damage from sharp instruments to the intact surface layer may occur.^{29,30} Non-destructive optical methods, such as quantitative light-induced fluorescence (QLF) and optical coherence tomography (OCT), can be utilized in conjunction with visual observation, which would give them the advantage of simplicity and ease of understanding.²¹

The ideal and most appropriate method for caries activity determination is through longitudinal assessment, where lesion progression is assessed multiple times over a period of time.¹⁶ As previously mentioned, there are some clinical signs to predict caries activity at the time of examination.^{12,31,32} Assessment of lesion activity in a single examination is difficult and requires training and calibration. Even with training, clinical assessment is based on the presence or absence of multiple risk parameters, and the examiner’s subjective opinion of their relative importance, as no single indicator is sufficient to assess activity alone.³¹

The visual appearance of non-cavitated early lesions perceived as dull or shiny serves as an indicator to caries lesion activity assessment.³³ Non-cavitated early lesions differ in color than the surrounding normal enamel due to the effect of increased porosity on the refractive index. Air drying removes water from pores, leaving only air in partially demineralized enamel, resulting in the white chalky appearance. Longstanding lesions in the oral cavity tend to pick up exogenous stains. One of the earliest investigations on browning of caries lesion in the 1950s centered on the amino-acids released during proteolysis during cavity formation (the “chelation theory”).³⁴ Driezen and Spies attributed the browning of tooth structure to melanin formation or a “Maillard reaction,” a non-enzymatic browning.³⁵ These pigments could form within the carious lesions, though external pigmentation is another possibility or co-factor. Uptake of food chromogens on the tooth surface or acquired pellicle into carious lesions through pores during the caries process has been demonstrated *in vivo* by Kidd et al.³⁶ Several studies discussed the validity of staining, especially marginal discoloration adjacent to existing restorations, in the diagnosis of secondary caries as it is not considered a valid criterion for the diagnosis of secondary caries and prediction of demineralization.³⁶⁻³⁸ Brown and yellow stains are an inconclusive variable in the detection and diagnosis of carious lesions; therefore, we attempted to eliminate the color variable from this study. Intrinsic and extrinsic chromogens fall under two categories: organic compounds with conjugated double bonds and metal-containing compounds. Bleaching performed by hydrogen peroxide or sodium hypochlorite involves an oxidation reaction with the double bonds resulting in a lighter color compound.³⁸ Bleaching of hypomineralized enamel lesions by sodium hypochlorite was effective clinically and *in vitro*.³⁷⁻³⁹

A few studies have suggested that the thickness of the enamel surface layer might be associated with caries activity.^{18,22,40,41} The current gold standard assessment methods, such as polarized light microscopy (PLM) and transverse microradiography (TMR), require destruction of the tooth specimen, rendering these methods unusable in clinical settings. Enamel surface porosity is suggested as the reason behind the change in QLF variables over time during the first few seconds of dehydration. QLF has been shown to discriminate between active and inactive lesions, but has not been correlated with surface layer thickness.⁴² Therefore, the main purpose of this study is to assess the surface layer thickness of active and inactive lesions and also determine if a correlation exists between surface layer thickness and caries activity.

SPECIFIC AIMS

The aims of this study are to:

1. Investigate if the average surface layer thickness measurement relates to visual/tactile caries activity assessment.
2. Investigate if there is an association between the thickness of the surface layer measured by μ CT and the caries activity determined with QLF during dehydration.
3. Investigate if visual and tactile activity assessments correlate with the values for surface roughness (Ra) measured by optical profilometry.
4. Determine the lesion severity by comparing lesion volume and maximum depth measured by μ CT.

HYPOTHESES

1. Visual/tactile (V/T) active caries lesions present thinner surface layers than V/T-inactive lesions.

Null Hypothesis (1): V/T active caries lesions do not present thinner surface layers than V/T inactive lesions.

2. There is an association between the surface layer thickness and activity determined with QLF during dehydration.

Null Hypothesis (2): There is no association between the surface layer thickness (measured with μ CT) and activity determined with QLF during dehydration.

3. Visual/tactile (V/T) active caries lesions present rougher surfaces than V/T-inactive lesions.

Null Hypothesis (3): V/T active caries lesions do not present rougher surfaces than V/T- inactive lesions.

REVIEW OF LITERATURE

VISUAL AND TACTILE EXAMINATION/INTERNATIONAL CARIES DETECTION AND ASSESSMENT SYSTEM (ICDAS)

Development of new technologies may provide supplemental benefits to clinical caries detection. These methods of assessment would only be meaningful if they can acquire measurements to quantify initial and subclinical lesions.¹¹ Early caries lesions amenable to preventive caries treatment are usually better detected by visual and tactile examination. Hence, the dental profession places a great reliance on meticulously exercised clinical visual/tactile caries examination, which requires assessment of lesion activity and surface integrity. In order to select the appropriate treatment course for caries intervention, it is necessary to distinguish between active and inactive, and between cavitated and non-cavitated lesions. The criteria in visual/tactile examinations focuses on those essential aspects stated in Nyvad's criteria,¹² which are widely recognized as suited for clinical practical settings. Nyvad's criteria of visual and tactile examinations are simple to use. Furthermore, they have provided a direct connection between the clinical presentation of the caries lesion and the treatment of choice. This has facilitated clinical caries-related treatment decisions by dental clinicians worldwide.⁶

The International Caries Detection and Assessment System (ICDAS) was first introduced in 2002 based on a systematic review of literature on caries detection systems published by Ismail⁴³ to provide a scientific evidence-based system.⁴⁴ The purpose of this system is to allow a standardized method of data collection in different clinical settings and better comparison grounds among studies. The net result is to provide a

better understanding of caries among different populations at an individual level.⁴⁴

ICDAS-I was devised in 2003 on the principle that visual and tactile examination should be carried out on a clean dry surface and sharp explorers are to be replaced with a ball-ended probe to avoid iatrogenic destruction of brittle enamel surfaces. Finally, ICDAS-II modification incorporated the introduction of codes that can reflect the increased severity of the caries lesions.⁴⁵ Several studies were conducted to substantiate the reproducibility and diagnostic accuracy of the ICDAS-II in caries detection at varying stages of disease progression.^{17,31,32,46-49} After a short period of training, one *in-vitro* study found a moderate to strong correlation with histological extents of the lesions along with improved sensitivity of visual examination among non-expert practitioners.⁴⁶ Another study showed good inter- and intra-examiner reproducibility and validity among non-trained examinations of occlusal caries.⁴⁷

Lesion activity assessment (LAA) depends on assembly of the knowledge gained from ICDAS determination of the lesion's clinical appearance and other criteria that may provide predictive value in activity status determination. Each criterion would receive a point and the summation of these points is judged at a cutoff point. A review³¹ indicated that if construct validity is accepted as a gold standard, it would be possible to assess lesion activity status at a single examination using information obtained from multiple indicators, such as visual appearance of the lesion, tactile sensation of the surface of the lesion, location of the lesion, and gingival health. Activity cannot be determined through a single predictor, but the summation of the knowledge gained from various predictors increases the accuracy of activity assessment in primary coronal and root lesions.³¹

Predictive validity of activity assessment associated with ICDAS criteria on primary teeth

was demonstrated by a two-year cohort study on occlusal lesions only, but not on smooth surfaces.¹⁷ Tikhonova et al.⁴⁸ published a study in 2014 comparing reproducibility and diagnostic outcome of ICDAS-II + LAA and Nyvad's criteria among non-experienced dental practitioners. Both systems showed high reproducibility in regard to lesion severity. A higher prevalence of active cavitated lesions with the ICDAS-II + LAA than those with the Nyvad system was noted. This finding is in agreement with a previous study reporting that ICDAS-II + LAA system overestimates active cavitated lesions when compared with Nyvad's criteria in primary teeth.³²

The ICDAS system has presented moderate to good performance in terms of sensitivity and specificity for occlusal lesions (0.63 to 0.82 and 0.63 to 0.94, respectively).^{45,46} As for proximal lesions, ICDAS has shown good inter-examiner reproducibility and a performance comparable to occlusal lesions *in vitro*.³² However, its sensitivity was low in *in-vivo* studies.⁴⁹ Although *in-vivo* studies on proximal surfaces are limited, the previous study supports the use of ICDAS + LAA system along with technology-based caries detection methods.⁴⁹

MICROCOMPUTED TOMOGRAPHY VALIDITY AS A GOLD STANDARD METHOD

X-ray computed tomography (CT) was first introduced in the early 1970s. It was successfully implemented in the practical field in 1972 by the English engineer G.N. Hounsfield. Advanced imaging technology has developed since then and revolutionized the practice of medicine. The images are collected from various angulations, and are then reconstructed, producing three-dimensional spatial distribution maps of the dense material within attenuated materials.⁵⁰ A conventional radiograph is limited to a two-

dimensional image, which superimposes the summation of material attenuation along the path of the x-ray. In contrast, a CT scan image produces a three-dimensional voxel image. Microcomputed tomography (μ CT) was developed in the early 1980s. Initially, the μ CT was developed for the purpose of detecting small defects in ceramic materials. μ CT has better resolution with a much smaller voxel size range of 5 μ m to 50 μ m, which is 1,000,000 times smaller than a regular CT scanner voxel.⁵¹ μ CT differs from a regular CT scanner in three aspects: 1) The specimen rotates rather than the x-ray source and detectors; 2) A two-dimensional detector is used to provide a direct three-dimensional reconstruction; and 3) It has a limited capacity of the imaged specimen dimensions.⁵¹

When μ CT scanners were first developed, they were custom-built and were not widely available. Nowadays, commercially compact systems are obtainable and are a valuable asset in both academic and industrial research laboratories.⁵² μ CT scanners can be utilized to view ceramics, polymers, biomaterial scaffolds, hard mineralized tissues, and soft tissues. The scanner is able to image soft tissue through contrast material perfusion. With the new generations of μ CT systems, some are able to image small live animals *in vivo* as well.⁵³ The analysis of μ CT images to estimate three-dimensional morphometric parameters of a material microstructure may be achieved through a direct or an indirect method.⁵⁴ The traditional method is based on assuming a particular underlying structure form indirectly estimated from two-dimension stereology equations. The novel approach is based on estimating the local parameters such as thickness by fitting spheres within the structures or between them at each image voxel. It measures the actual distances in three-dimensional space directly.⁵⁴

μ CT has been used to depict mineral density in different tooth structures. One study used μ CT to measure the mineral density of white spot enamel lesions.⁵⁵ The mineral densities of sound enamel and white spot lesions with non-cavitated surfaces were from $2.65 \text{ g}\cdot\text{cm}^{-3}$ to $2.89 \text{ g}\cdot\text{cm}^{-3}$ and $2.23 \text{ g}\cdot\text{cm}^{-3}$ to $2.58 \text{ g}\cdot\text{cm}^{-3}$ respectively. The lowest levels of density of white spot lesions were from $1.48 \text{ g}\cdot\text{cm}^{-3}$ to $2.03 \text{ g}\cdot\text{cm}^{-3}$. Five different hydroxyapatite phantoms were used for calibration during each scan.⁵⁵ The accuracy of μ CT imaging was compared with measurements of 12 teeth made by a direct caliper, a 3D scanner, and by photography.⁵⁶ The results of this study suggested that μ CT is a reliable method for linear measurements and a valuable tool for observing internal and external tooth structure.⁵⁶ Hamba et al.⁵⁷ and Soviero et al.⁵⁸ confirmed the high level of sensitivity and specificity of μ CT relative to the gold standard methods of histology and transverse microradiography. Three dimensional measurements of mineral loss and lesion depth in natural lesions obtained from μ CT with a combination of Al and Cu filters agreed with transverse microradiography (TMR) parameters, thus validating this technique as an effective alternative for non-destructive quantitative demineralization and remineralization laboratory studies.⁵⁷ When proximal surfaces in primary molars were examined, μ CT showed the highest accuracy, highest values of sensitivity, and highest correlation with histology (0.88; 0.86) when compared with ICDAS and bitewing radiographs, followed by ICDAS (0.83; 0.74). Based on these results, Soviero et al.⁵⁸ advocated the use of μ CT as a gold standard for detecting caries lesions in proximal surfaces in primary molars.

An *in-vitro* study published in 2016 measured the thickness of the surface layer of enamel caries lesions. Linear scans of the mineral content profile were used across the

sidewalls and the floor of fissures and at the central traverse of the proximal lesions.²⁷

The average thickness of surface layer in fissural lesions is considerably higher than smooth surface proximal lesions. This study demonstrated the efficacy of μ CT for high-resolution non-destructive study of fissural lesions.²⁷ A recent study confirmed the accuracy of ICDAS criteria and demonstrated the feasibility of using a quantitative μ CT approach as gold standards for the presence of carious lesions.⁵⁹

QUANTITATIVE LIGHT-INDUCED FLUORESCENCE

Quantitative light-induced fluorescence (QLF) is an optical method that objectively assesses the change in mineral content of smooth surface non-cavitated early enamel lesions. QLF depends on the analysis of backscattering fluorescence emitted by the enamel. When wavelengths at 400 nm are used, caries appears as a dark spot with the excite fluorescence at wavelengths above 520 nm.⁶⁰ The use of laser fluorescence with appropriate filters enhances the contrast between non-cavitated early enamel lesions and sound enamel *in vitro*.⁶¹ Subsequent investigations led to the development of a quantitative method of assessment of demineralization *in vitro*. Several studies established the relation between mineral loss and altered optical properties of enamel.⁶²⁻⁶⁵ A number of clinical studies were carried out to validate the application of QLF for clinical early caries detection. Ogaard et al.⁶² conducted an *in-vivo* study where smooth surface lesions were induced on vital premolars of orthodontics patients. Lesions were imaged weekly for four weeks following the removal of orthodontic bands. It was noted that lesions regressed very rapidly after the causal factor (orthodontic bands) was eliminated. The study established that QLF can be used for clinical quantitative non-destructive caries assessment. In 1997 Alkhateeb et al.⁶⁵ evaluated the effect of fluoride

remineralization treatments on enamel lesions. They were able to detect the amount of enamel remineralization that occurred with each fluoride regimen through longitudinal assessment. These results correlated with results obtained from microradiography.

Since these studies, QLF has typically been used to monitor the progression of caries lesions in longitudinal studies.⁶⁶ One study investigated the effect of fluoride varnish on non-cavitated early enamel lesions using QLF in a randomized clinical trial.⁶⁷ The study validated the use of QLF as a sensitive clinical method suitable for longitudinal quantification of non-cavitated early enamel lesions on smooth surfaces.⁶⁷ QLF was also compared with other modalities of caries detection and existing “gold standard” such as TMR, laser fluorescence (DIAGNODENT®), and electronic caries monitor (ECM). All of the mentioned studies found a favorable correlation between fluorescence loss and enamel demineralization depth.^{65,68-70} However, it should be noted that the use of QLF should be combined with visual and tactile examinations, given that QLF is unable to distinguish the difference between non-cavitated early enamel lesions and other non-carious enamel hypomineralizations.⁷¹ QLF tends to show a higher sensitivity compared with other methods, specifically the ICDAS visual and tactile examinations; this is attributed to the QLF analysis software, which detects any change in pixel values as demineralization, thereby compromising its specificity. Using a combined methodology with ICDAS has proved its efficacy in allowing earlier detection of lesions that are more likely to progress as demonstrated in a longitudinal study.⁷²

In 2013 Ferreira et al.⁷³ published a four-year longitudinal clinical study of baseline QLF variables compared at regular intervals. They found that regardless of the ICDAS score at baseline, lesions that were more likely to progress showed a rapid rate of

increase in QLF variables versus the lesions that did not progress. This indicates the ability of QLF to monitor changes in lesion severity and to differentiate between active and inactive lesions over time.⁷³ Another retrospective study found an association between high levels of red/orange fluorescence and lesion progression to cavitation.⁷⁴ The increase in red fluorescence emission in QLF images could be the result of excitation of endogenous porphyrins. Therefore, early detection of red/orange fluorescence can be a predictive indicator of caries activity.

Several studies have discussed the effect of dehydration on QLF, showing that fluorescence backscattering of demineralized enamel decreases gradually when specimens are dehydrated at room temperature for 30 minutes or longer.^{75,76} Ando et al.⁴² studied the effect of dehydration on extracted teeth with non-cavitated early lesions on QLF variables. They concluded that, in the first few seconds of dehydration by continuous compressed air, active lesions showed a rapid decrease in light absorption and fluorescence remittance. The authors traced the cause of such changes to the existence of enamel microchannels larger than those found in inactive lesions. The study suggested that the change in QLF variables during the first few seconds of dehydration can differentiate between active and inactive caries lesions at the time of examination.⁴² The same authors demonstrated similar results in a recent clinical pilot study.⁷⁷ Twenty-four white spot enamel lesions on the buccal surfaces of 23 children were evaluated. Three consecutive images were acquired by QLF at baseline, 5 seconds, and 15 seconds. Images acquired from both active and inactive lesions were compared with each other as visual/tactile examination was considered the gold standard. Although the results were not statistically significant, they suggested that QLF during dehydration has the potential

to distinguish between active and inactive non-cavitated enamel lesions at the time of examination.⁷⁷

Another recent *in-vitro* study evaluated the reflection, roughness, and fluorescence change in demineralization/remineralization models.⁷⁸ Enamel samples were subjected to multiple species microbial demineralization and pH cycling remineralization. Measurements of sound, demineralized, and remineralized surfaces were obtained for reflection using reflectometry, for roughness by profilometry, and for fluorescence by QLF. The results confirmed that active lesions are associated with less light reflection and thus appear dull, while inactive lesions show greater light reflection and appear shinier. As for QLF activity assessment, the change in ΔQ per second (ΔQ_D) was significantly greater in all three demineralization groups than in the remineralization groups. Both demineralization and remineralization groups had greater ΔQ_D than the sound group. This demonstrated the ability of QLF during dehydration to determine caries lesion activity at the time of the examination.⁷⁸

MATERIALS AND METHODS

SAMPLE SELECTION AND PREPARATION

One hundred extracted human teeth were collected from dental practitioners in the state of Indiana and stored in 0.1-percent thymol solution. The collection of human teeth for use in dental laboratory research studies has been approved by the Indiana University (IU) Institutional Review Board (IRB) (IRB# NS0911-07). Extracted human premolars with approximal smooth surface non-cavitated early enamel lesions surrounded by sound enamel were selected for the study. Teeth were cleaned of extrinsic debris with a soft brush and water. As explained in the section “Gaps of Knowledge,” stains are an inconclusive variable in the detection and diagnosis of carious lesions. We attempted to eliminate the color variable from this study. Therefore, after bleaching with 5.0-percent NaOCl for 30 minutes, the teeth were washed under running water and stored in 0.1-percent thymol to remove any stains that could cause bias in specimen collection. A pilot study was performed beforehand to establish the effect of bleaching and bleaching time on non-cavitated early enamel lesion roughness. Five extracted human premolars with active smooth surface non-cavitated caries lesions surrounded by sound enamel, as well as five teeth with inactive smooth surface non-cavitated lesions were selected based on visual and tactile examination. The enamel lesions measured approximately $2 \times 2 \text{ mm}^2$. Specimens were subjected to profilometric scanning before and after bleaching with 5.0-percent NaOCl for 2 hours. The pilot study implied that surfaces of active enamel lesions would present higher surface roughness than inactive enamel lesions. Bleaching increased the surface roughness of enamel lesions, but not considerably.

Approximal non-cavitated early lesions selected for the study measured approximately $2 \times 5 \text{ mm}^2$ and were not stained. Caries lesion severity was assessed following the criteria of the International Caries Detection and Assessment System (ICDAS).⁴³ Only teeth exhibiting ICDAS scores of 1 and 2 were included in the study. Teeth were assessed using ICDAS criteria by two experienced examiners (M.A, F.A.) by consensus with the aid of an overhead light unit and air syringe. Teeth were assessed moist first, then dried and examined after 5 seconds. Enamel lesions with visible enamel surface defects were excluded. The apical one-third of the root was reduced, leaving the coronal two-thirds, using diamond discs (Lapcraft's L'il Trimmer™, Powell, Ohio, USA). Next, the teeth were mounted with the use of superglue on Lego® bricks separately to standardize the location and angulation of the lesions. Teeth were stored in 100-percent humidity, with a wet cotton pellet over the tested surfaces, in numbered containers at 4°C.

VISUAL AND TACTILE EXAMINATION

Conventionally, caries activity is determined through longitudinal assessment as lesions progress or arrest. Based on Nyvad et al.^{12,13} and Ekstrand et al.³¹ longitudinal clinical studies demonstrated that cross-sectional visual/tactile examinations would associate with caries activity. Therefore, visual/tactile examinations criteria can be used to speculate caries activity at the time of examination. For a cross-sectional study, such as this study, activity assessment was designated as visual-tactile caries activity.

TRAINING

Visual/tactile examinations were conducted by consensus by two experienced examiners (K.D., A.S.). Examiners were trained as they attended a lecture on the diagnostic criteria of active and inactive non-cavitated early enamel lesions. They were also trained to distinguish surface roughness using two metal plate surfaces (Average roughness [R_a]: 0.13 μm and 2.04 μm). The area examined was limited to the site of the lesion at a $2 \times 5 \text{ mm}^2$ window. A #6 Handle-Satin Steel explorer (23 SE, Hu-Friedy Mfg. Co., Inc., USA) was held at 45° to the examined surface and moved as passively as possible. Each examiner measured the amount of force exerted on the explorer. The amount of force was not to exceed 20 gm (0.1961N). All collected extracted teeth with ISDAS scores of 1 or 2 were examined.

SPECIMEN SELECTION

Specimens were air-dried using compressed air for at least 30 seconds. Examination was carried out with the aid of a dental unit light and an air syringe. A scale was used before any examination to standardize the amount of force exerted by the explorer during examination. Each examiner measured the amount of force exerted on the explorer prior to the initiation of examination and after the examination of 50 teeth. The amount of force did not exceed 20 gm (0.1961N). Examiners evaluated both the appearance (visual) and the roughness (tactile) of each specimen. They surveyed the indicated area and determined the surfaces as smooth or rough, dull or shiny, and assessed the caries activity as active or inactive.

The lesions were divided into active and inactive categories. Active non-cavitated enamel lesions are opaque and show a chalky, matte surface. The surface texture is rough when the tip of a probe is moved gently across the surface. Inactive non-cavitated enamel lesions may look whitish or brownish-black due to staining; however, only whitish lesions were included. They are shiny/glossy and feel smooth on gentle probing. The examiners reported their findings with the aid of a facilitator. Fifteen visually active caries lesions and 15 visually inactive caries lesions were selected by consensus for the study.

SURFACE OPTICAL PROFILOMETRY

To measure the roughness of lesion surfaces, enamel lesions and sound smooth surfaces in each specimen were scanned with a computer-guided optical profilometer (Proscan 2000A; Scantron Industrial Products Ltd., Taunton, UK). This method uses a 50-watt halogen bulb as a light source. The light is focused onto the measured surface as a spot approximately 4 μm in diameter. The scanning frequency was 300 Hz, with a step size of 0.01 mm for the x-axis (parallel to long axis of plates) and of 0.01 mm for the y-axis (perpendicular to long axis of plates), and a resolution of 0.01 μm . The S38/3a sensor was used. An area of 2×3 mm² was scanned per lesion and per sound smooth surface. The profilometric data were digitally recorded and processed by dedicated software (Proscan 2000 version 2.0.17; Scantron). In this study, the parameters were limited to the arithmetic mean roughness R_a . A single scanning profile was recorded for each specimen and an average R_a was acquired. An auto level filter, a size 2 surface filter and a size 1 warpage filter were used to level the scanned surface. (R_a lesion - R_a sound) / R_a sound was calculated for each specimen.

MICROFOCUS COMPUTED TOMOGRAPHY (μ CT) IMAGING

μ CT [Skyscan 1172 Micro-CT (Skyscan 1172, Aartselaer, Belgium)] was used to determine the severity of lesions and the thickness of the surface layer. A set of μ CT images was obtained using 80 kV, 134 μ A, 8 μ m pixel size resolution, and 0.5mm Al/40 μ m Cu filter to reduce beam hardening effects; a rotation step of 0.2°, and frame average of 4 were used. Reconstruction was done using Feldkamp algorithm by Skyscan's volumetric NRecon® reconstruction software. Smoothing was set at 7, beam hardening at 33, and ring artifact correction at 9. The images were set to view the entire crown of the tooth. Image processing was done using CT Analyzer software (Bruker micro CT, Kontich, Belgium). A μ CT threshold was constructed through calculating a grayscale by averaging 8 to 9 pixel-values at three points (upper, lower and middle of each enamel and dentin lesion) along each scan using Adobe®Photoshop®. Gray scale less than 95 percent of sound enamel in the surfaces of the region of interest along the sagittal slices indicated a caries lesion. The volumes and the areas of caries lesion variables were determined from μ CT Imaging 3D reconstruction to obtain the severity of caries based on a previous finding where active caries were both smaller in volume and more porous than inactive lesions.²⁶

Another set of μ CT images was obtained using 80 kV; 134 μ A; 5- μ m pixel size resolution; 0.5mm Al/40 μ m Cu filter to reduce beam hardening effects; a rotation step of 0.2°, and a frame average of 4. Reconstruction was done using Feldkamp algorithm by Recon online. Smoothing was set at 7, beam hardening at 33, and ring artifact correction at 9. The imaging was focused on the enamel surface with the non-cavitated early lesions on view only. Image processing was done also using CT Analyzer software (CTAn)

(Bruker micro CT, Kontich, Belgium). The area of interest was marked manually on each reconstructed scan image. The 2D and 3D morphometric analyses of the surface layer thickness were done using a special task list in CTAn software and region of interests were at 27 to 255 threshold. The thickest, thinnest, and average surface layer thicknesses were extrapolated from the image analyzer software task manager.

QUANTITATIVE LIGHT-INDUCED FLUORESCENCE IMAGING (QLF) DURING DEHYDRATION

The method followed is described in a previous study by Ando et al.⁴²

Fluorescence imaging was acquired using a QLF machine in a dark room. The light source was fixed to provide optimum illumination for the tested surface area. Specimens were exposed to 13 mW/cm² of violet blue light (WL 290 nm to 450 nm). Images were acquired through a 520-nm high-pass filter, transmitting light at a wavelength ≥ 250 nm. Specimen hydration was standardized by placing a wet cotton pellet saturated in distilled water on the enamel surface for 60 seconds. The cotton pellet was removed prior to acquiring images. A baseline image was acquired immediately after removal of the cotton pellet and a series of images were acquired at 1s, 5s, and 15s,⁴² during which specimens were dehydrated by continuous compressed air of 36 psi and an airflow of 8.5 l/m from a 3-cm fixed distance. QLF variables (average fluorescence loss [%], size [mm²], ΔQ [% \times mm²] and the change-in-QLF-variables per second [ΔQ_D]) were determined. The change in QLF variables indicated the activity, while the score at 15s correlated the severity of the lesion.

STATISTICAL ANALYSES

Two-sample t-tests were used to compare the profilometry surface roughness (Ra); Ra lesion/Ra sound, Ra lesion - Ra sound, (Ra lesion - Ra sound)/ Ra sound , μ CT lesion bone volume, μ CT gray values for sound tissue, μ CT lesion depth, μ CT surface layer thickness (3D, 2D, 2D-average from individual data points, 2D-minimum from individual data points, 2D-maximum from individual data points), QLF ΔQ , and QLF change in ΔQ (ΔQ_D) per second of dehydration between teeth with and without visual indications of caries activity. Scatterplots and Pearson correlation coefficients were used to evaluate the associations of QLF ΔQ at 15 seconds dehydration with lesion depth and lesion bone volume and the associations of QLF ΔQ_D per second of dehydration with surface roughness and surface layer thickness. A 5.0-percent significance level was used for all tests.

SAMPLE SIZE JUSTIFICATION

With a total sample size of 30 teeth, 15 each with or without visual indications of caries activity, the study will have 80-percent power to detect a correlation of 0.49.

Additionally, 15 specimens each with or without visual indications of caries activity will have 80-percent power to detect a difference of 16 μ m between groups, assuming a standard deviation of 15 μ m and comparisons using a two-sample t-test at a 5.0-percent significance level.

RESULTS

VISUAL AND TACTILE EXAMINATION

Visual and tactile examination of non-cavitated early lesions on the proximal surfaces of 100 teeth to determine activity yielded 48 active and 52 inactive lesions. Forty-six of the active lesions were dull and rough, two were dull and smooth. Forty-eight of the inactive lesions were considered to be dull and smooth, while only four were considered shiny and smooth. No statistical comparisons were performed due to the limited number of surfaces that were determined to be shiny (four surfaces total) (Table III).

SURFACE ROUGHNESS (Ra) (μm)

Table IV and Figure 6 present the mean and standard deviation of surface roughness (Ra) between teeth with and without visual indications of caries activity. Two-sample t-tests showed no statistically significant difference in surface roughness between active and inactive lesions. Ra values of sound surfaces were $1.27 (\pm 0.20) \mu\text{m}$ and $1.24 (\pm 0.24) \mu\text{m}$ ($p = 0.739$) respectively for active and inactive lesions. Table IV and Figure 7 present $(\text{Ra lesion} - \text{Ra sound})/\text{Ra sound}$ between teeth with and without visual indications of caries activity. $(\text{Ra lesion} - \text{Ra sound})/\text{Ra sound}$ was $0.26 (\pm 0.55) \mu\text{m}$ for active lesions and $0.22 (\pm 0.40) \mu\text{m}$ for inactive lesions ($p = 0.839$) showing no statistically significant difference.

SURFACE LAYER THICKNESS

Table V and Figure 5 present the mean and standard deviation of surface layer thickness for both active and inactive non-cavitated early lesions. The thinnest surface layer measured 25 μm and the thickest measured 92 μm . Both values were within the active lesion surface layer. The average 2D surface layer thicknesses of active and inactive lesions were 57 μm and 52 μm respectively. Active and inactive non-cavitated early lesions did not have significantly different surface layer thickness. A two-sample t-test was performed on values acquired from 2D ($p = 0.121$) and 3D morphometric analyses ($p = 0.080$) of μCT images, 2D average surface thickness ($p = 0.446$), as well as 2D average of the minimum value of surface thickness ($2D_{\text{Min}}$) ($p = 0.197$) and 2D average of the maximum value of surface thickness ($2D_{\text{Max}}$) ($p = 0.122$).

GRAY VALUES

Gray values (representing μCT thresholds) for sound tissue of active and inactive teeth were compared. Active and inactive non-cavitated early lesions did not have significantly different gray values for sound tissue (Table VI; two-sample t-test; $p = 0.178$ for dentin and 0.655 for enamel).

LESION DEPTH (μm)

Lesion depth in dentine was significantly larger for active lesions (Table VII; $p = 0.009$); however, active and inactive lesions did not have significantly different enamel or total lesion depth ($p = 0.111$ for enamel and 0.132 for total lesion) Figure 2.

LESION VOLUME (mm³)

Enamel and overall lesion volumes (mm³) from μ CT image analysis were significantly larger in active lesions than in inactive lesions; however, there was no significant difference in dentin lesion volume between active and inactive lesions (Table VIII; Figure 1, two-sample t-test, $p = 0.075$ for dentine lesion volume, 0.031 for enamel lesion volume, and 0.022 for overall lesion volume). A 0.95- μ CT threshold was utilized in the analyses.

QLF ΔQ AT 15 SECONDS OF DEHYDRATION (ΔQ_{15})

Active and inactive non-cavitated early lesions did not have significantly different ΔQ_{15} (% \times mm²) in all QLF thresholds tested (Table IX; two-sample t-test; $p = 0.199$ at 5% threshold, 0.186 at 10%, 0.17 at 15%, 0.138 at 20%, 0.101 at 25% and 0.100 at 30%) (Figure 3).

QLF OF DEHYDRATION (ΔQ_D)

Table X and Figure 8 present ΔQ_D (% \times mm²/sec) of dehydration in active and inactive non-cavitated early lesions. ΔQ_D (% \times mm²/sec) of dehydration was significantly larger for active lesions only for 1-second dehydration at 5.0-percent threshold ($p = 0.046$). Active and inactive lesions did not have significantly different ΔQ /sec of dehydration for any other dehydration time (Table X; two-sample t-test, $p = 0.347$ at 2 s, 0.077 at 3 s, 0.080 at 4 s, 0.097 at 5 s, 0.091 at 10 s, 0.120 at 15 s).

ASSOCIATIONS OF ΔQ_D WITH SURFACE ROUGHNESS

ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) was not significantly associated with surface roughness using scatterplots and Pearson correlation coefficients (PCC). At QLF threshold 5.0 percent and 15 seconds of dehydration $\text{PCC} = 0.03$ ($p = 0.879$) (Table XI).

ASSOCIATIONS OF ΔQ_D WITH MICRO-CT SURFACE LAYER THICKNESS

At QLF 5.0-percent threshold, ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-min were significantly correlated, with weak negative associations at 1 s, 3 s, 5 s, 10 s, and 15 s of dehydration; at 15 seconds of dehydration $\text{PCC} = -0.40$ ($p = 0.026$). Similarly, ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-avg were significantly correlated, with a weak negative association at QLF 5.0 percent threshold with 15 seconds dehydration ($\text{PCC} = -0.36$; $p = 0.049$). ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) was not significantly associated with surface layer thickness 3D (all $p \geq 0.188$), surface layer thickness 2D (all $p \geq 0.083$), or surface layer thickness 2D-max (all $p \geq 0.106$) (Table XII; Figures 9,10).

ASSOCIATIONS OF ΔQ WITH MICRO-CT LESION DEPTH AND LESION VOLUME

Associations were performed using scatterplots and Pearson correlation coefficients. QLF ΔQ at 15 seconds of dehydration (ΔQ_{15}) was not significantly associated with μCT lesion depth at any QLF thresholds tested (Table XIII; all $\text{PCC} \leq 0.31$; all $p \geq 0.097$). For ΔQ_{15} at 5.0-percent threshold, $\text{PCC} = 0.22$ ($p = 0.225$). ΔQ_{15} and μCT lesion volume were significantly correlated, with a moderate positive association regardless of QLF threshold. (all $\text{PCC} \geq 0.56$; all $P \leq 0.001$). At 0.95 μCT threshold and ΔQ_{15} , $\text{PCC} = 0.56$ ($p = 0.001$) (Table XIV; Figures 11,12).

ASSOCIATIONS OF SURFACE ROUGHNESS WITH MICRO-CT SURFACE LAYER THICKNESS

Surface roughness was not significantly associated with μ CT surface layer thickness. PCC was 0.12 for 3D SLT ($p = 0.529$), 0.07 for 2D ($p = 0.729$), 0.00 for 2D-avg ($p = 0.994$), -0.24 for 2D-min ($p = 0.209$), -0.05 for 2D-max ($p = 0.796$) (Table XV).

TABLES AND FIGURES

TABLE I

ICDAS criteria for the study teeth selection

ICDAS code	Description
ICDAS code 1	Teeth that show the first visual change in enamel; opacity or discoloration (white or brown) is visible at the surface of white spot lesion (WSL) after prolonged air-drying, which is not or hardly seen on a wet surface.
ICDAS code 2	Teeth with distinct visual change in enamel, opacity, or discoloration distinctly visible at the surface of WSL under dry condition, lesion is visible when wet.

TABLE I

ICDAS caries lesion activity assessment*

ICDAS Code	Characteristics of Lesion	
	Active Lesion	Inactive Lesion
1, 2 or 3	Surface of enamel is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved gently across the surface. Lesion is in a plaque stagnation area, i.e. pits and fissures, near the gingival and approximal surface below the contact point.	Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface. For smooth surfaces, the caries lesion is typically located at some distance from the gingival margin.

Specimen #	Visual		Tactile		Activity	
#	Shiny	Dull	Smooth	Rough	Active	Inactive

*During V/T examination the examiners reported the findings with the aid of the facilitator.

TABLE II

Analysis of visual and tactile evaluations to determine caries activity

Activity	Dull	Shiny	Rough	Smooth	Dull- Rough	Dull- Smooth	Shiny- Smooth
Active	48 (50%)	0 (0%)	46 (100%)	2 (4%)	46 (100%)	2 (4%)	0 (0%)
Inactive	48 (50%)	4 (100%)	0 (0%)	52 (96%)	0 (0%)	48 (96%)	4 (100%)

TABLE IV
Comparisons between teeth with and without visual
indications of caries activity and surface roughness Ra (μm)

	Active	Inactive	p-value
Ra lesion	1.57 (0.65)	1.51 (0.51)	0.761
Ra Sound	1.27 (0.20)	1.24 (0.24)	0.739
(Ra lesion - Ra sound) / Ra sound	0.26 (0.55)	0.22 (0.40)	0.839

*Active and inactive specimens did not have significantly different surface roughness.

TABLE V

Comparisons between Two-sample t-test, Mean (\pm SD) surface layer thickness (mm) of teeth with and without visual indications of caries activity

Type	Active (SD)	Inactive (SD)	p-value
3D	0.082 (0.023)	0.069 (0.015)	0.080
2D	0.049 (0.013)	0.042 (0.008)	0.121
2D-avg	0.057 (0.023)	0.052 (0.010)	0.446
2D-min	0.025 (0.012)	0.030 (0.007)	0.197
2D-max	0.092 (0.038)	0.075 (0.016)	0.122

*Active and inactive specimens did not have significantly different surface layer thickness.

TABLE III

Two-sample t-test, comparisons between teeth with and without visual indications of caries activity and gray values for sound tissue*

Enamel/Dentin	Active (SD)	Inactive (SD)	p-value
Dentine	37.8 (0.6)	37.1 (1.2)	0.178
Enamel	60.6 (1.4)	60.8 (1.3)	0.655

*Active and inactive specimens did not have significantly different gray values for sound tissue.

TABLE IV

Two-sample t-test, comparisons between teeth with and without visual indications of caries activity and lesion depth (μm)

Enamel/Dentin	Active (SD)	Inactive (SD)	p-value	
Dentine	374 (252)	156 (159)	0.009	*
Enamel	389 (150)	466 (97)	0.111	
Total	763 (298)	622 (192)	0.132	

*Lesion depth in dentine was significantly larger for active specimens, but active and inactive specimens did not have significantly different enamel or total lesion depth.

TABLE V

Two-sample t-test, Mean (\pm SD) lesion volume (mm³)
in teeth with and without visual indications of caries activity

Enamel/Dentin	μ CT Threshold	Active (SD)	Inactive (SD)	p-value	
Dentine	0.95	0.14 (0.20)	0.03 (0.04)	0.075	
Enamel	0.95	0.26 (0.16)	0.15 (0.07)	0.031	*
Total	0.95	0.38 (0.31)	0.17 (0.09)	0.022	*

*Lesion volume was significantly larger for active lesions (enamel volume and overall volume).

TABLE IX

Two-sample t-test, mean (\pm SD) change in fluorescence (ΔQ) after 15 seconds dehydration in teeth with and without visual indications of caries activity and QLF ΔQ at 15 seconds dehydration.

QLF Threshold			
(%)	Active (SD)	Inactive (SD)	p-value
5	131 (87)	97 (54)	0.199
10	119 (86)	84 (52)	0.186
15	98 (84)	63 (46)	0.174
20	74 (76)	40 (36)	0.138
25	49 (61)	20 (26)	0.101
30	25 (38)	7 (15)	0.100

TABLE VI

Two-sample t-test, Mean (\pm SD) change in fluorescence [Δ QD ($\% \times \text{mm}^2/\text{sec}$)] (QLF 5% threshold) after varying dehydration times in teeth with and without visual indications of caries activity

QLF Threshold	Dehydration (sec)	Active (SD)	Inactive (SD)	p-value	
5	1	11.6 (12.5)	3.2 (9.6)	0.046	*
	2	7.0 (9.1)	4.0 (7.7)	0.347	
	3	8.0 (8.8)	3.0 (5.5)	0.077	
	4	7.0 (7.6)	2.5 (5.8)	0.080	
	5	5.6 (6.0)	2.3 (4.5)	0.097	
	10	4.1 (4.9)	1.5 (2.9)	0.091	
	15	2.8 (3.2)	1.2 (2.6)	0.120	

TABLE VII

Pearson correlation coefficients of associations of QLF ΔQ_D
 ($\% \times \text{mm}^2/\text{sec}$) with surface roughness R_a (μm)*

QLF Threshold (%)	Dehydration (sec)	Pearson correlation	p-value
5	1	0.05	0.781
	2	0.04	0.854
	3	-0.03	0.863
	4	-0.03	0.887
	5	-0.09	0.657
	10	-0.01	0.939
	15	0.03	0.879
10	1	0.12	0.547
	2	0.06	0.747
	3	-0.01	0.959
	4	-0.01	0.966
	5	-0.08	0.673
	10	0.00	0.983
	15	0.03	0.867
15	1	0.21	0.272
	2	0.13	0.483
	3	0.04	0.836
	4	0.04	0.833
	5	-0.04	0.833

(continued)

TABLE VIII (cont.)

20	15	0.09	0.645
	1	0.15	0.426
	2	0.07	0.729
	3	0.04	0.850
	4	0.02	0.905
	5	-0.02	0.925
	10	0.03	0.861
	15	0.09	0.638
25	1	0.06	0.754
	2	0.03	0.889
	3	0.02	0.927
	4	0.00	0.995
	5	-0.04	0.854
	10	0.01	0.954
	15	0.05	0.792
30	1	-0.04	0.835
	2	0.00	0.983
	3	-0.01	0.961
	4	-0.04	0.838
	5	-0.05	0.784
	10	0.00	0.984
	15	0.00	0.984

* QLF ΔQ_D (% \times mm²/sec) was not significantly associated with surface roughness.

TABLE XII

Pearson correlation coefficient of association of QLF ΔQ_D
 ($\% \times \text{mm}^2/\text{sec}$) with μCT surface layer thickness

Surface Layer Thickness	QLF Threshold	Dehydration (sec)	Pearson Correlation	p-value
3D	5	1	0.06	0.775
		2	-0.06	0.757
		3	-0.10	0.597
		4	-0.22	0.253
		5	-0.20	0.280
		10	-0.16	0.410
		15	-0.25	0.188
2D	5	1	-0.07	0.710
		2	-0.14	0.463
		3	-0.19	0.307
		4	-0.30	0.107
		5	-0.28	0.130
		10	-0.22	0.255
		15	-0.32	0.083
2D-avg	5	1	-0.27	0.156
		2	-0.11	0.578
		3	-0.21	0.265
		4	-0.23	0.225

(continued)

TABLE XII (cont.)

		5	-0.26	0.161	
		10	-0.24	0.204	
		15	-0.36	0.049	*
2D-min	5	1	-0.61	<.001	*
		2	-0.21	0.264	
		3	-0.43	0.016	*
		4	-0.32	0.085	
		5	-0.39	0.031	*
		10	-0.37	0.046	*
		15	-0.40	0.026	*
2D-max	5	1	-0.25	0.191	
		2	-0.09	0.645	
		3	-0.14	0.461	
		4	-0.17	0.378	
		5	-0.19	0.309	
		10	-0.18	0.332	
		15	-0.30	0.106	

*QLF ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-min/ 2D-avg were significantly correlated, with a weak negative association.

TABLE IX

Pearson correlation coefficients of associations of QLF ΔQ with μ CT lesion depth (15 seconds dehydration)*

QLF Threshold (%)	Pearson Correlation	p-value
5	0.22	0.251
10	0.22	0.254
15	0.23	0.228
20	0.26	0.160
25	0.31	0.097
30	0.30	0.109

*QLF ΔQ was not significantly associated with μ CT lesion depth.

TABLE X

Pearson correlation coefficients of associations of QLF
 ΔQ with μCT lesion volume

μCT Threshold	QLF Threshold (%)	Pearson Correlation	p-value	
0.95	5	0.56	0.001	*
	10	0.56	0.001	*
	15	0.56	0.001	*
	20	0.57	0.001	*
	25	0.58	0.001	*
	30	0.56	0.001	*

*QLF ΔQ and μCT lesion volume were significantly correlated, with a moderate positive association.

TABLE XI

Pearson correlation coefficients of associations of surface roughness with μ CT surface layer thickness

Surface Roughness	Surface Layer Thickness	Pearson correlation	p-value
Ra	3D	0.12	0.529
	2D	0.07	0.729
	2D-avg	0.00	0.994
	2D-min	-0.24	0.209
	2D-max	-0.05	0.796

Surface roughness was not significantly associated with μ CT surface layer thickness.

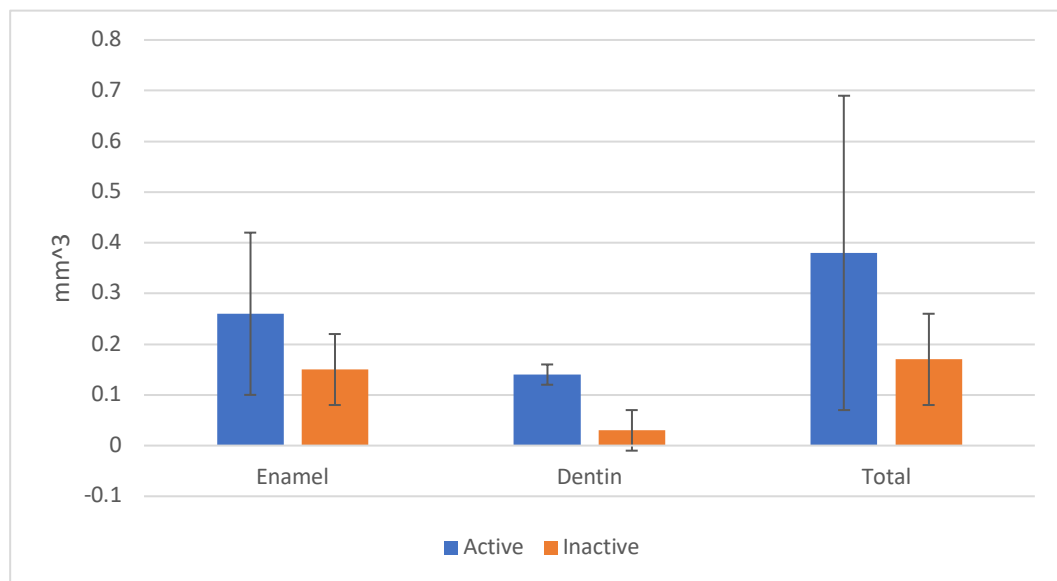


FIGURE 1. Lesion volume (mm³) in teeth with and without visual indications of caries activity.

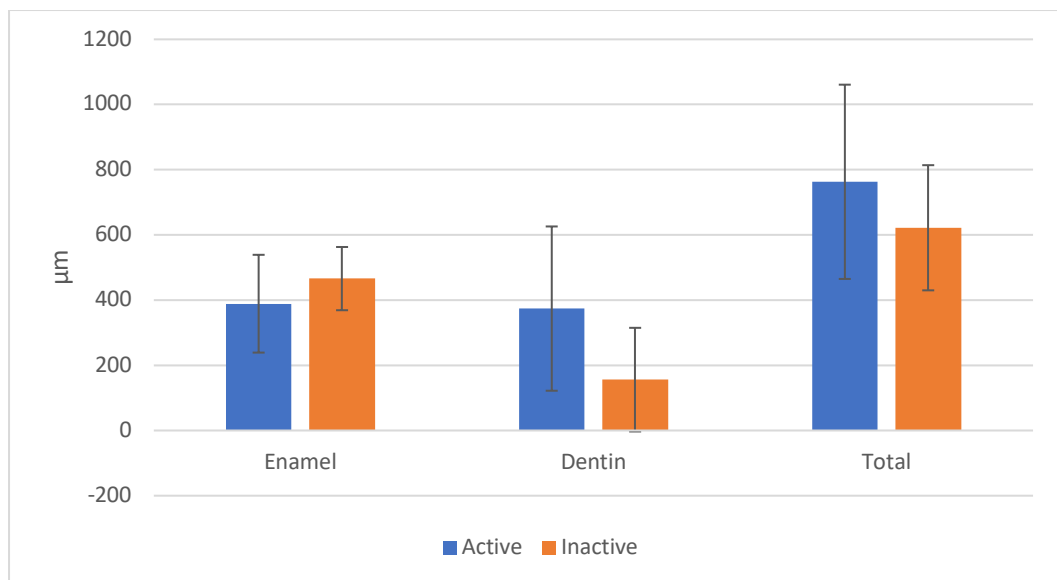


FIGURE 1. Lesion depth (μm) in teeth with and without visual indications of caries activity.

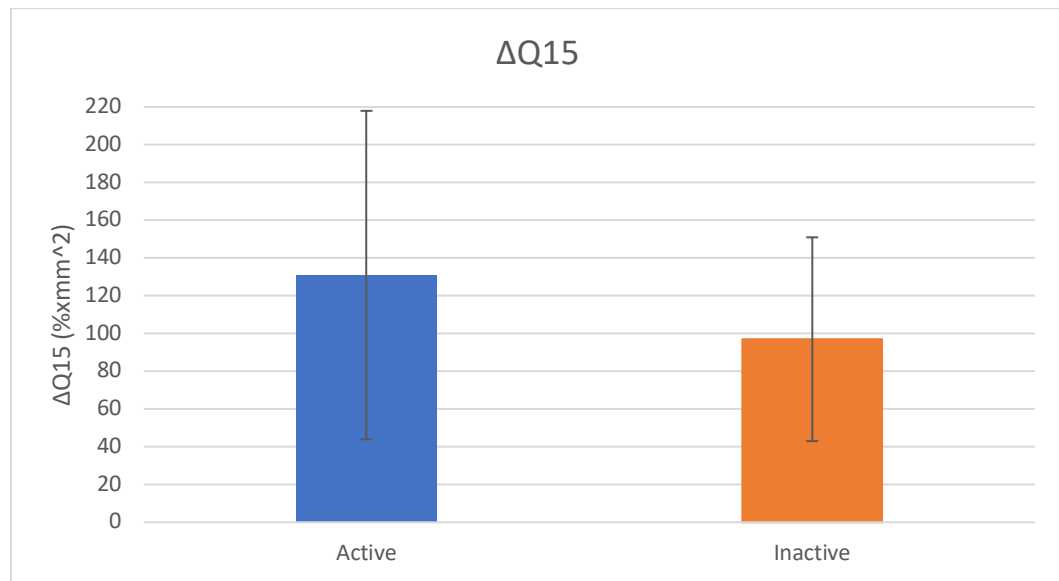


FIGURE 3. Comparisons between teeth with and without visual indications of caries activity and QLF ΔQ at 15 seconds of drying.

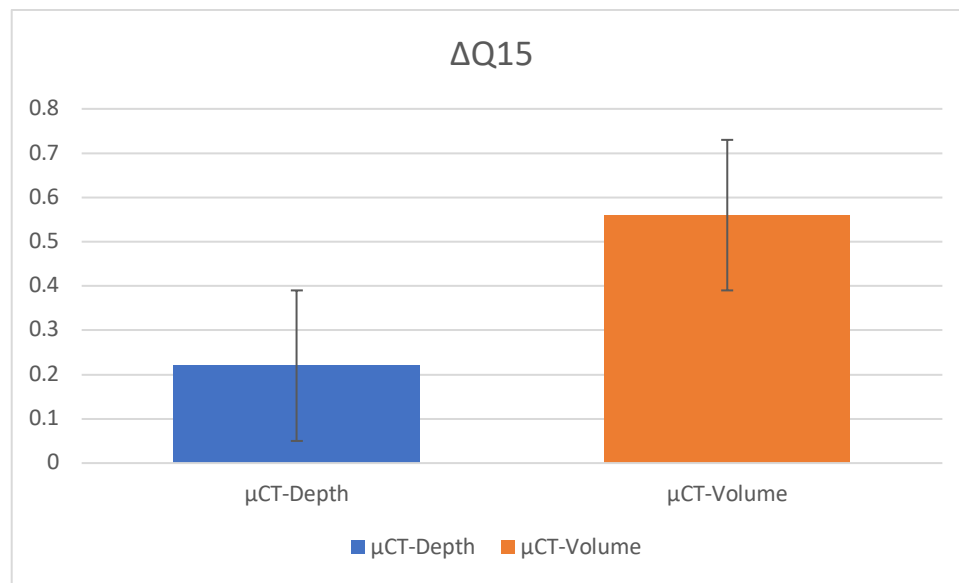


FIGURE 2. Associations of QLF ΔQ at 15 sec of dehydration with μCT lesion depth and lesion volume (Pearson correlation coefficient).

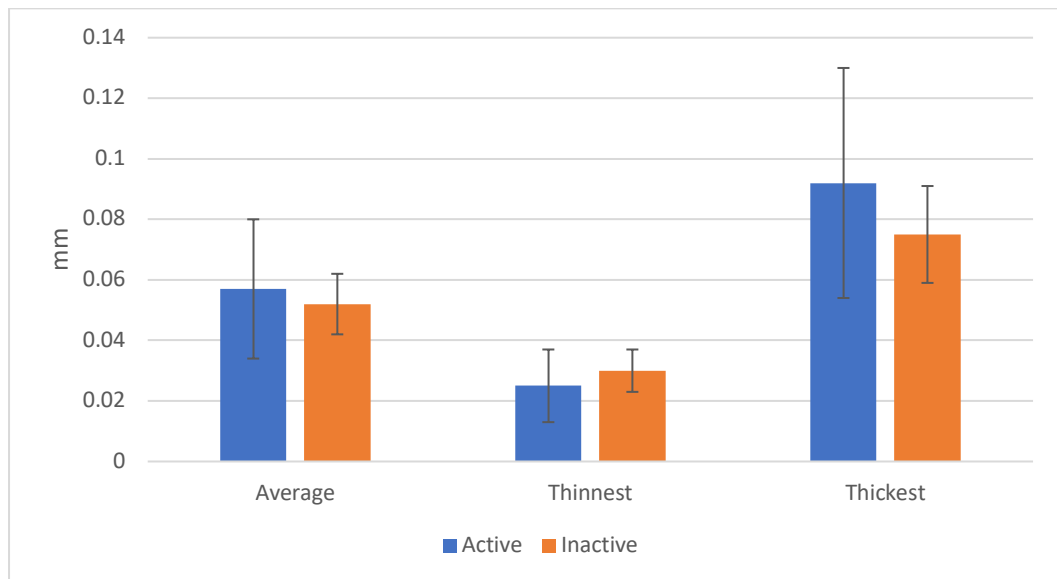


FIGURE 3. Comparisons between teeth with and without visual indications of caries activity and surface layer thickness (mm).

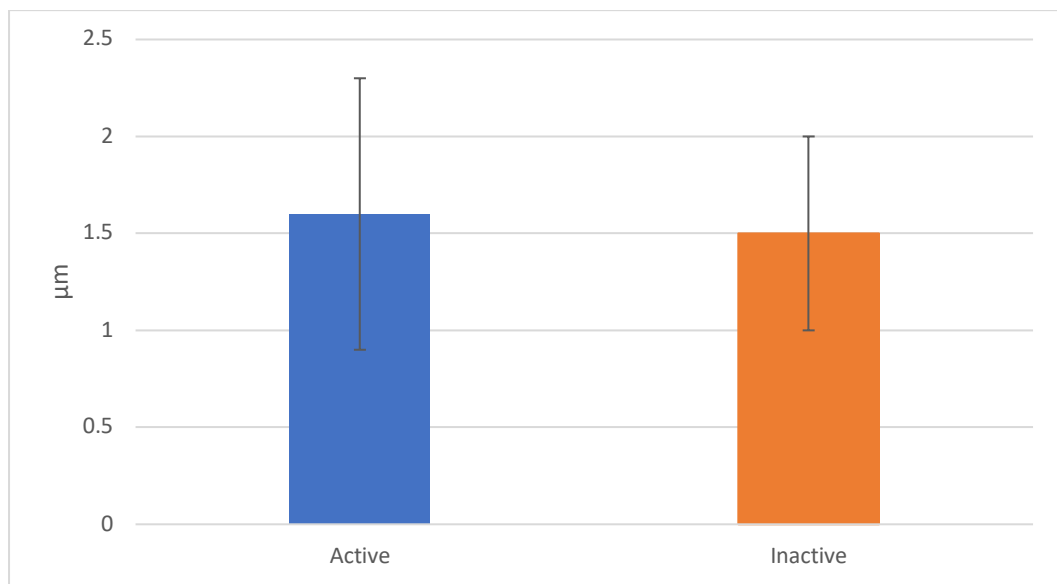


FIGURE 6. Comparisons between teeth with and without visual indications of caries activity and surface roughness Ra (μm).

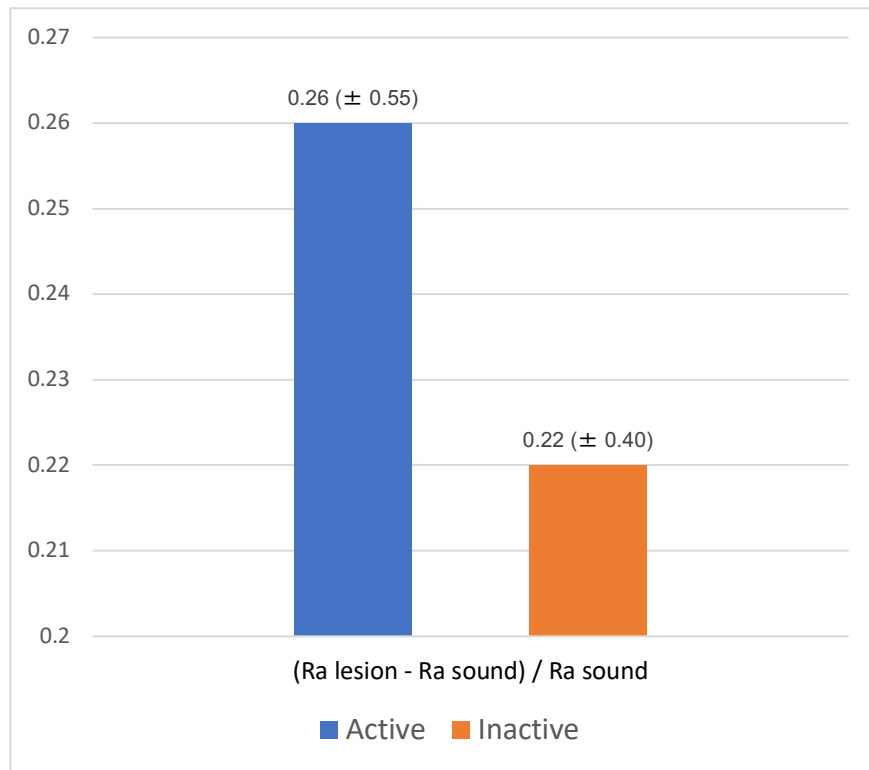


FIGURE 7. Comparisons between teeth with and without visual indications of caries activity surface roughness Ra, $(Ra_{\text{lesion}} - Ra_{\text{sound}}) / Ra_{\text{sound}}$.

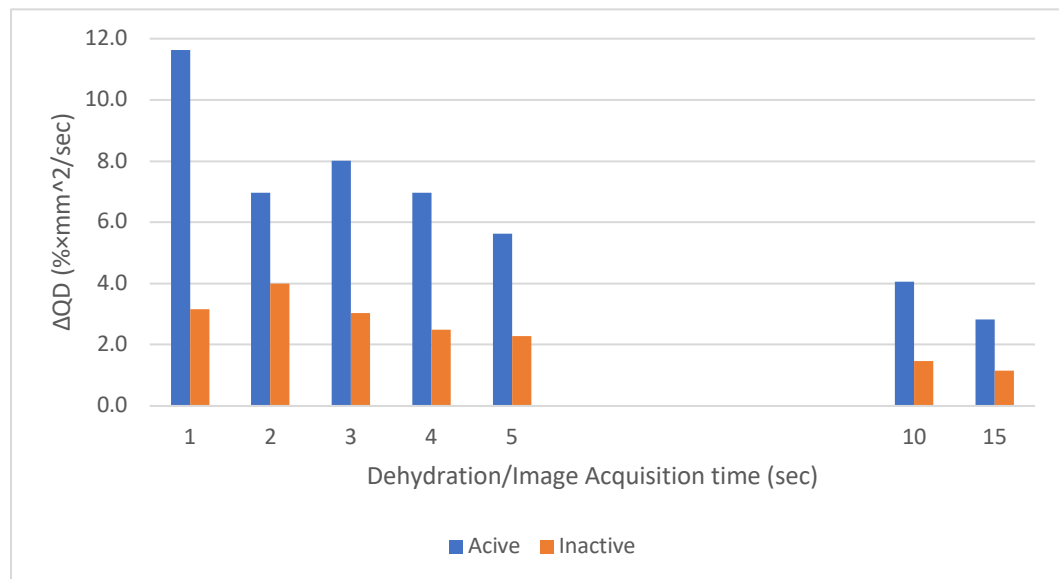


FIGURE 4. Comparisons between teeth with and without visual indications of caries activity and ΔQ_D ($\% \times \text{mm}^2/\text{sec}$).

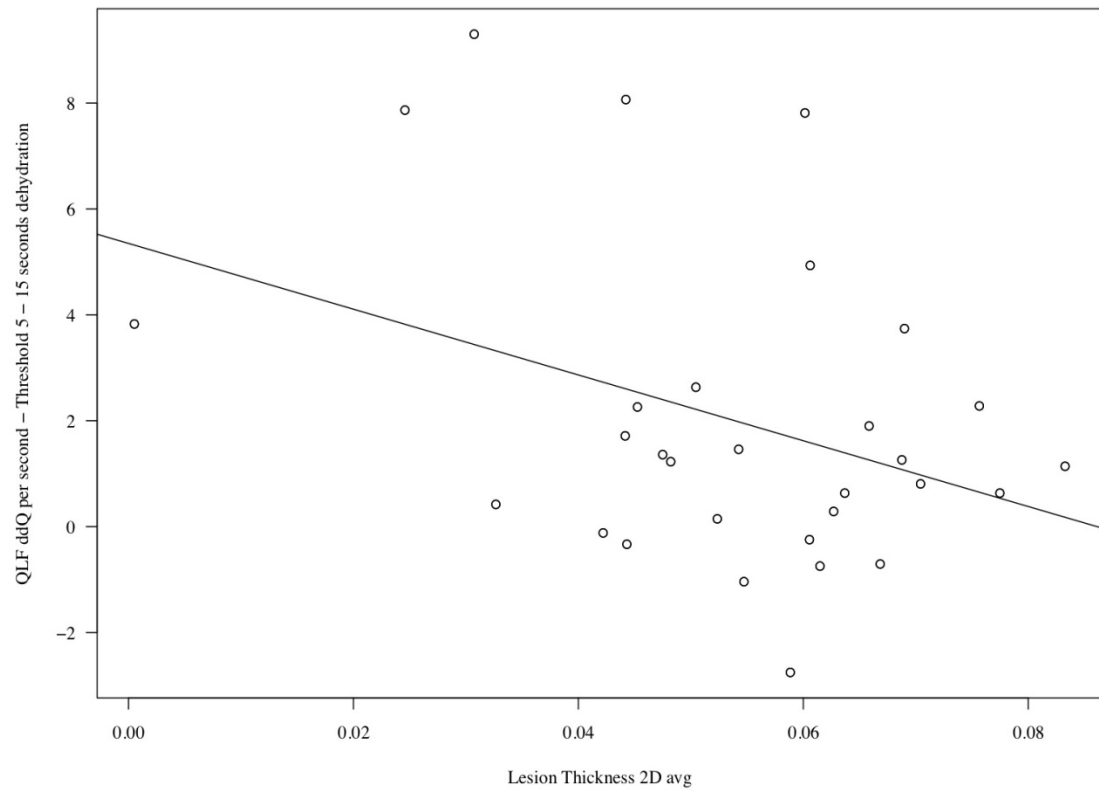


FIGURE 5. Scatterplot of correlation of QLF ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-avg. *2D-avg were significantly correlated, with a weak negative association.

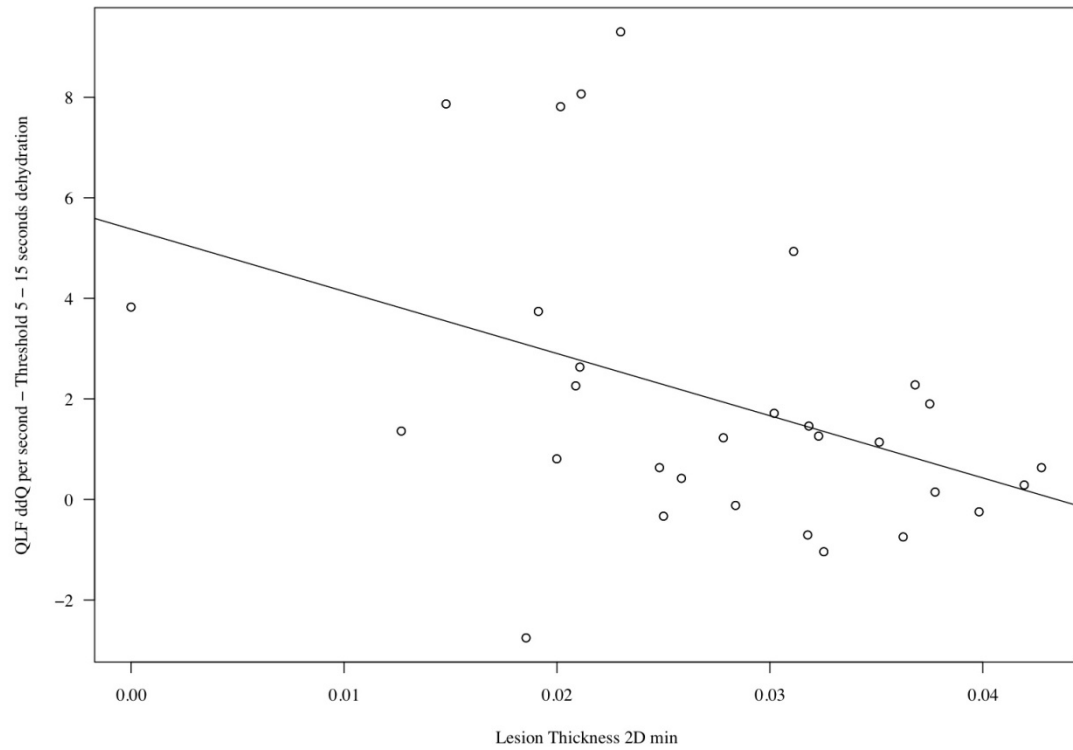


FIGURE 6. Scatterplot of correlation of QLF ΔQ_D (% \times mm²/sec) and surface layer thickness 2D-min. *2D-min were significantly correlated, with a weak negative association.

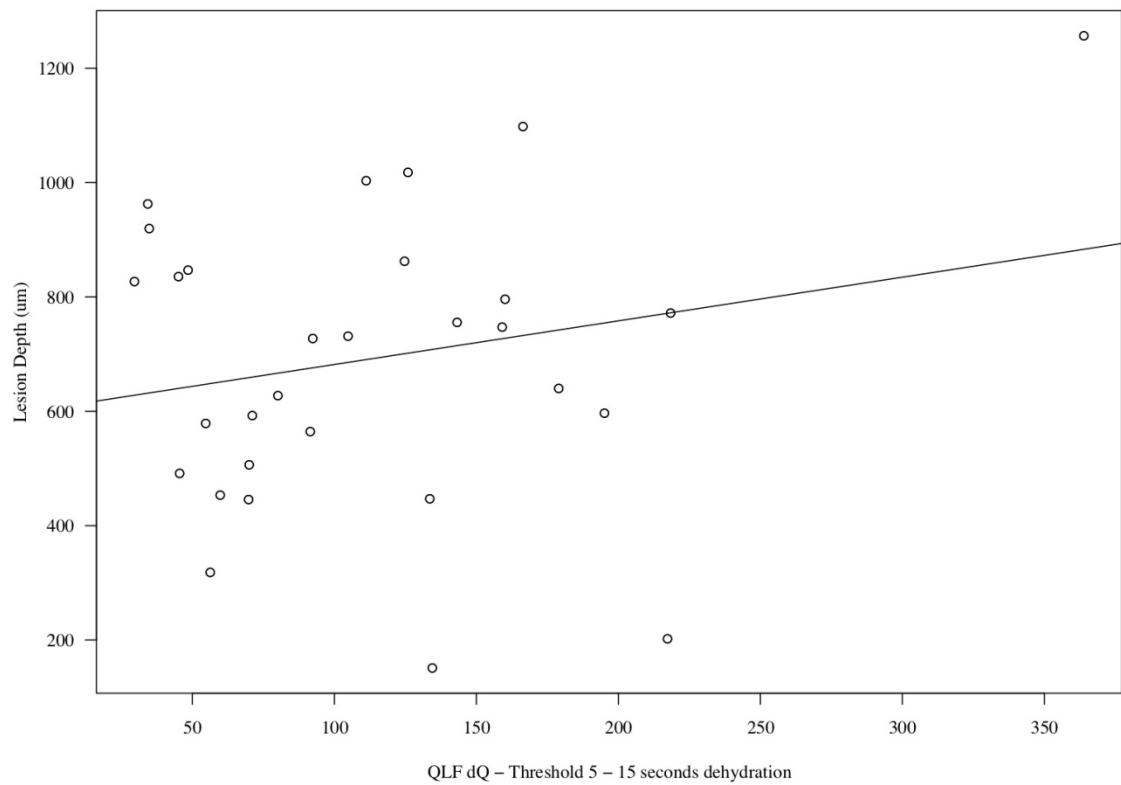


FIGURE 7. Scatterplot of association of QLF ΔQ with μCT lesion depth (μm). QLF ΔQ is not significantly associated with μCT lesion depth.

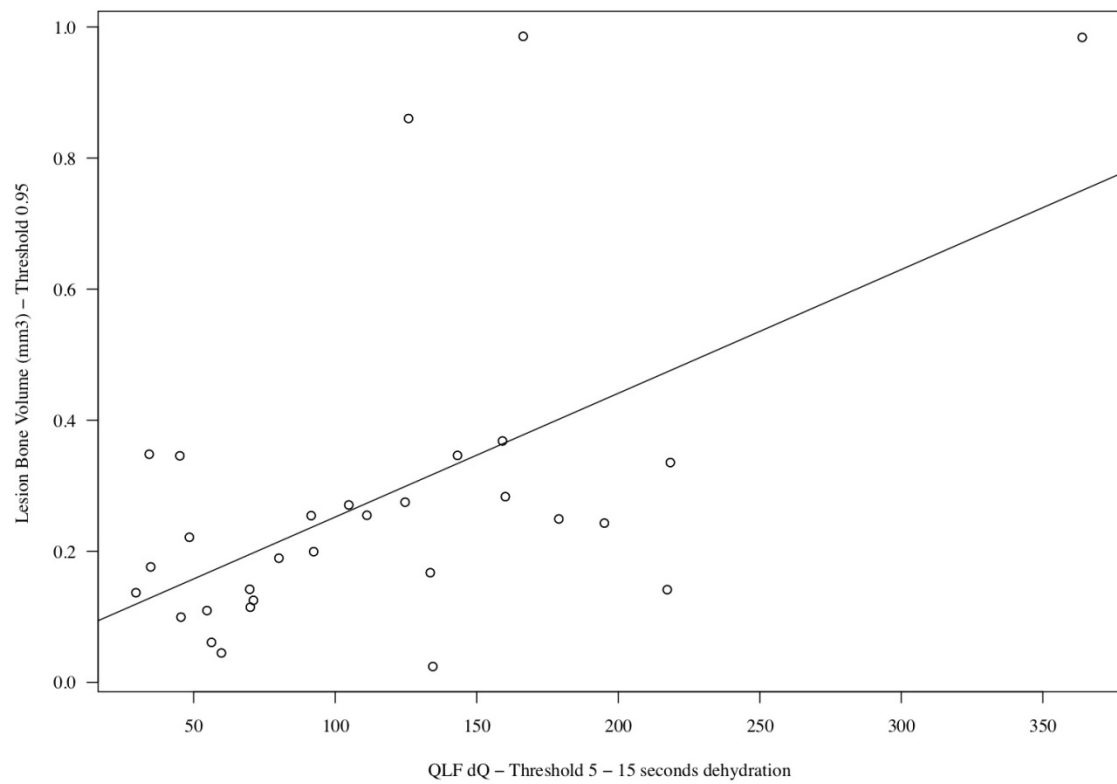


FIGURE 8. Scatterplot of association of QLF ΔQ with μ CT lesion volume (mm³) significantly correlated with a moderate positive association.



FIGURE 9. Sample preparation on Lego® bricks.

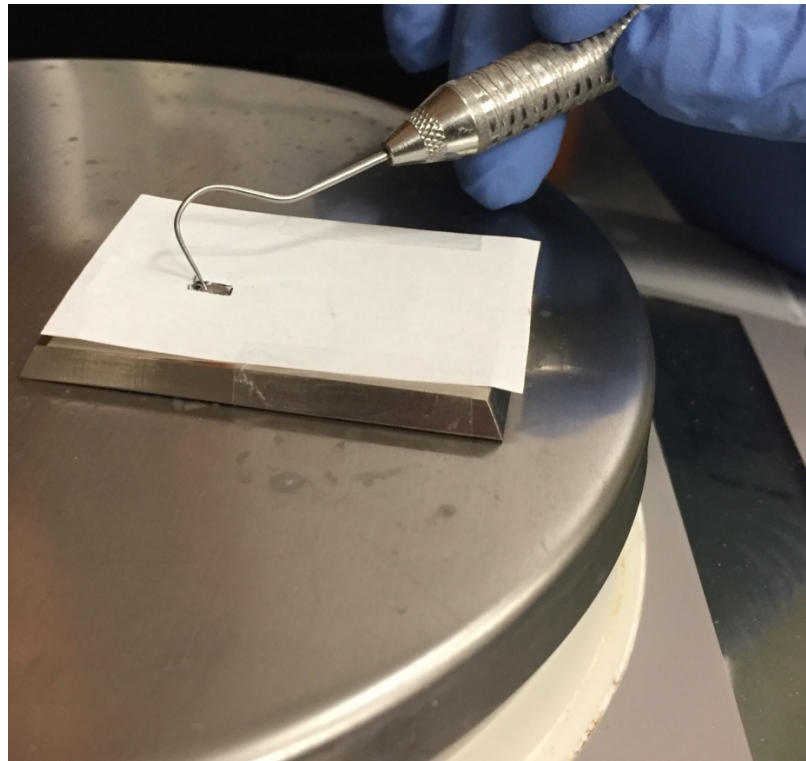
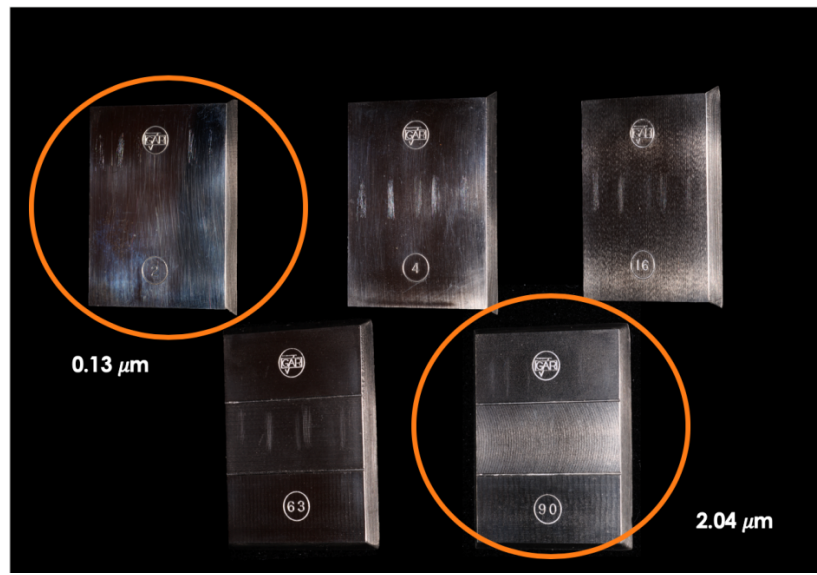


FIGURE 10. Metal plates used for training with a 2×5mm window opening and a scale to measure the amount of force exerted while probing.

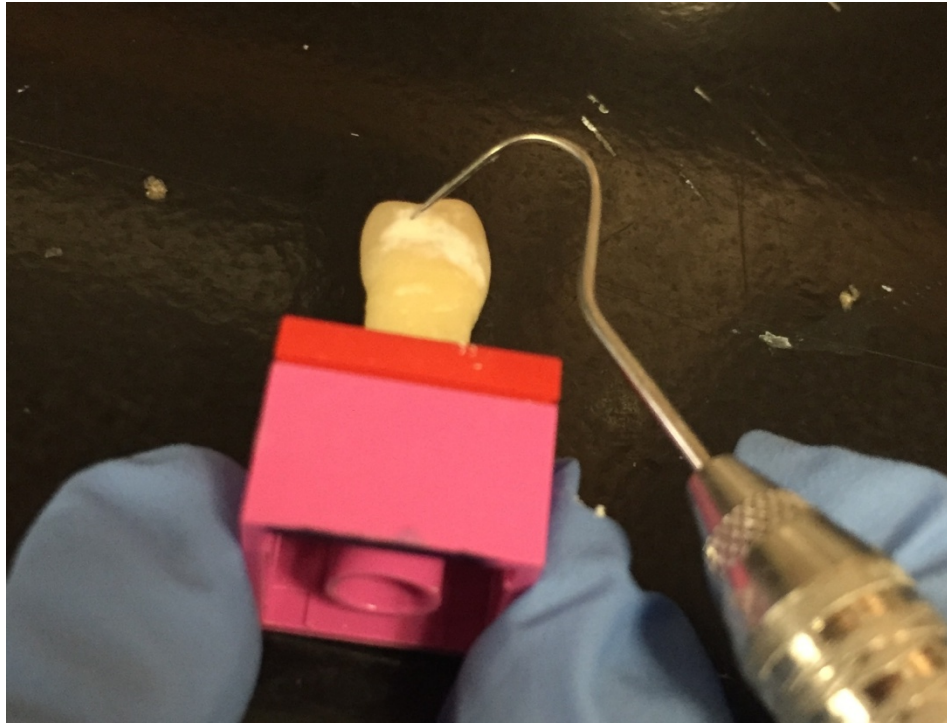


FIGURE 11. Tactile examination using a #6 Handle Satin Steel explorer (23 SE, Hu Friedy Mfg. Co., Inc., USA).

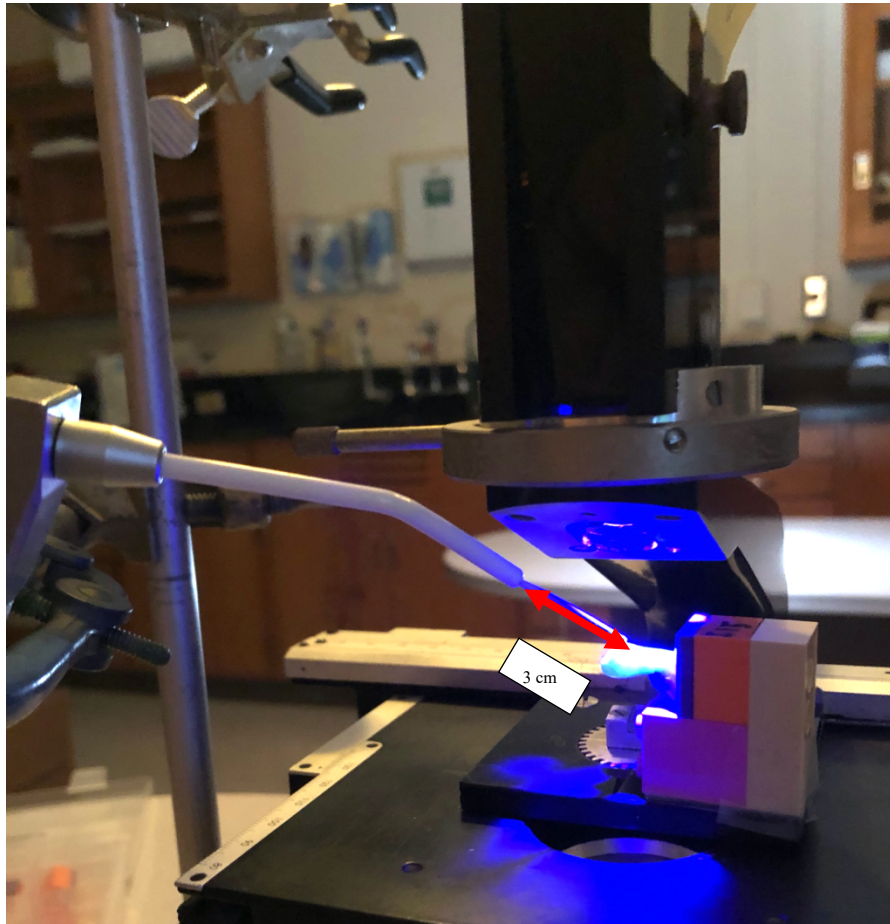


FIGURE 12. QLF during dehydration assembly.

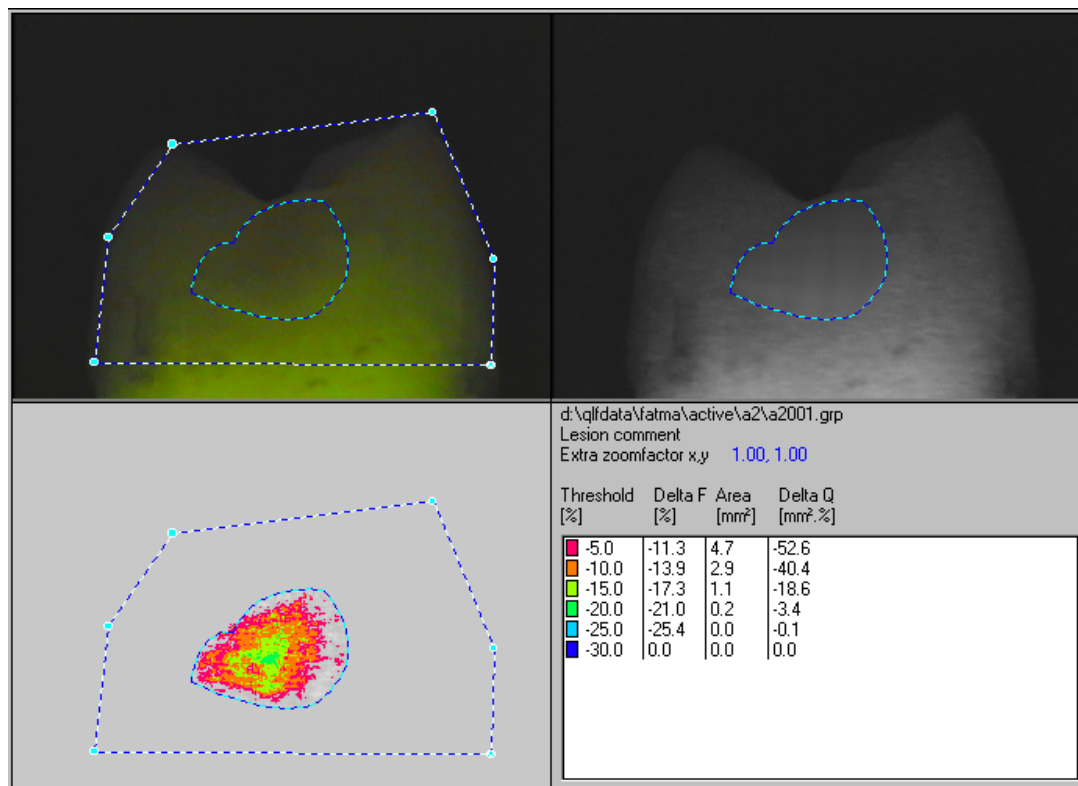


FIGURE 13. QLF image analysis.

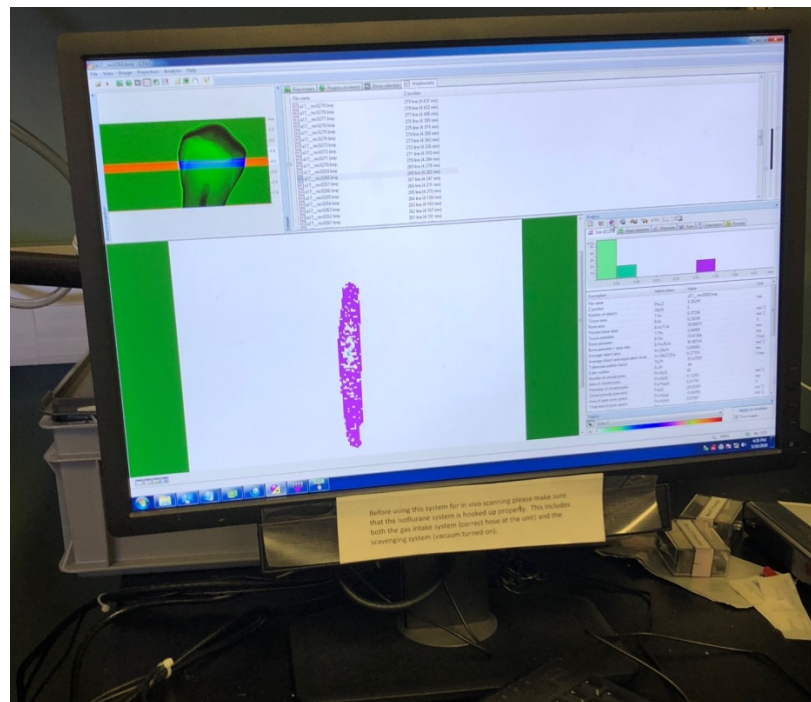
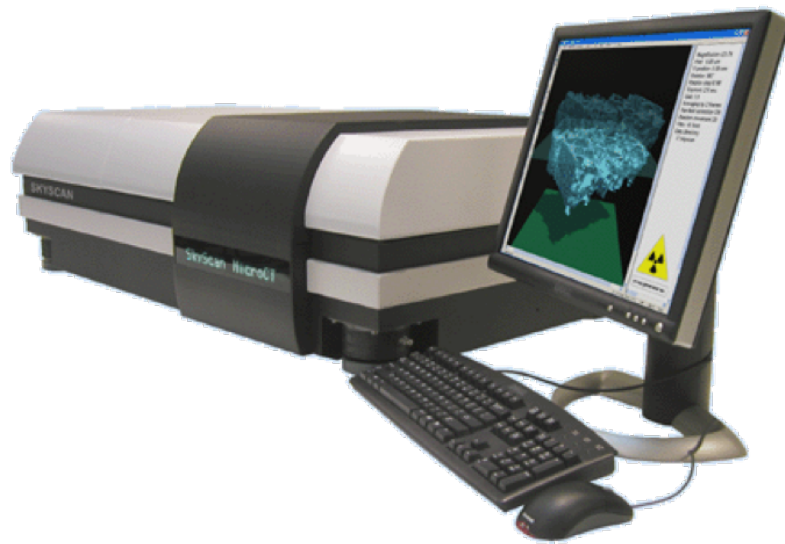


FIGURE 14. Micro CT imaging and analysis.

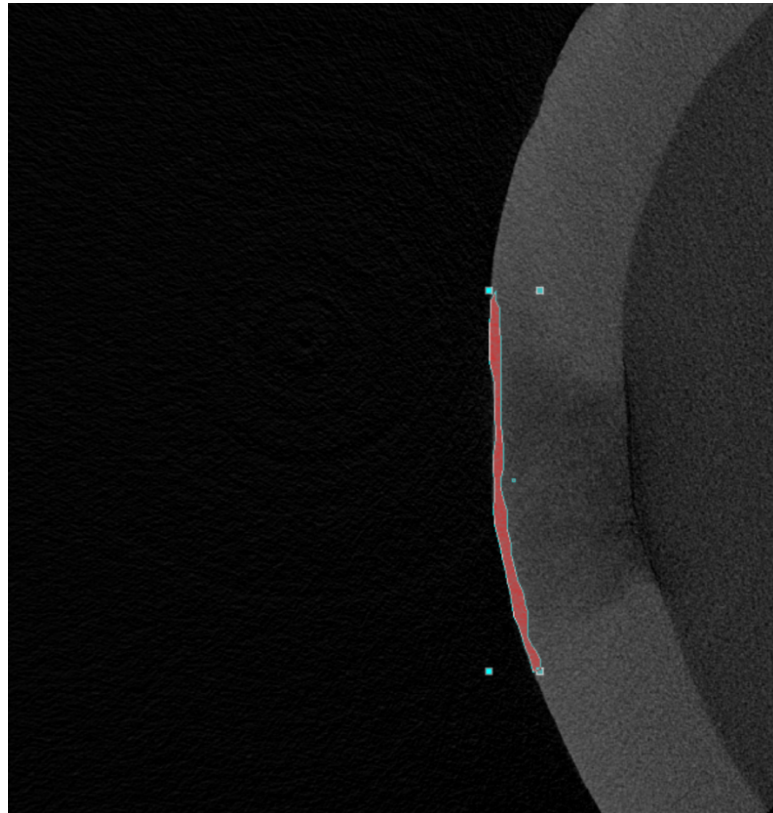


FIGURE 19. The region of interest function in CTAn software was used to isolate the surface layer in enamel lesions.

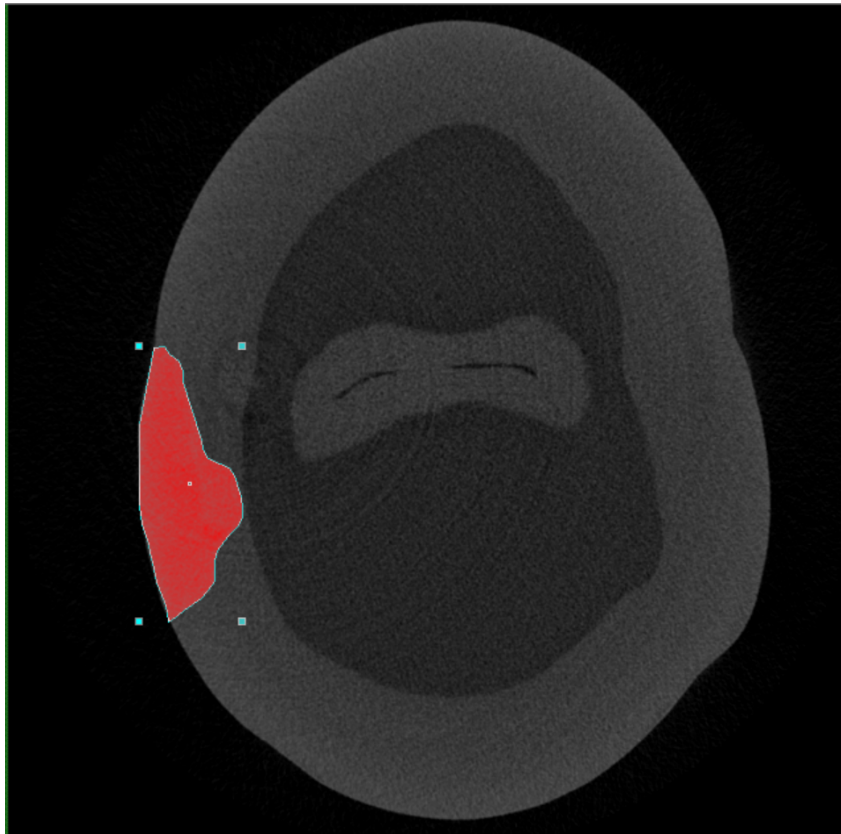


FIGURE 15. The region of interest function in CTAn software was used to isolate the enamel lesions.

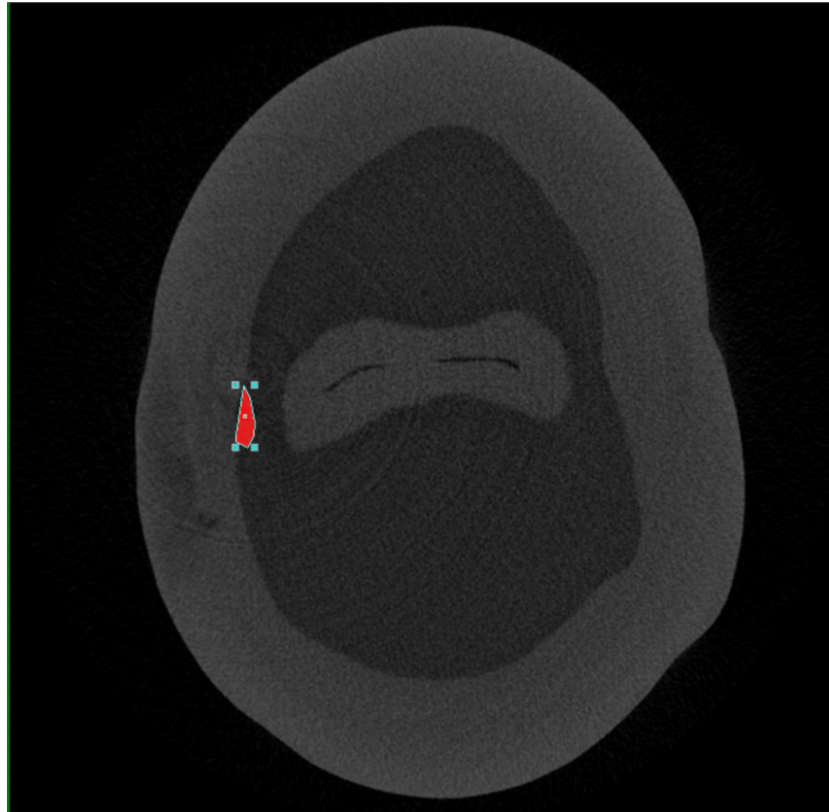


FIGURE 16. The region of interest function in CTAn software was used to isolate the dentin lesions.

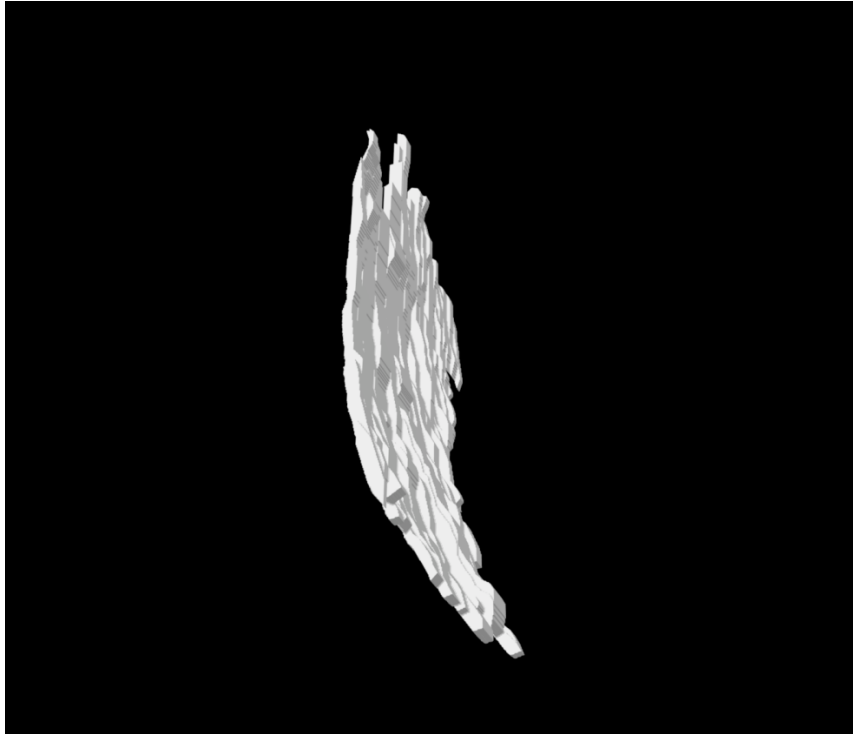


FIGURE 17. 3D reconstruction of surface layer of non-cavitated white spot enamel lesion from μ CT image analysis.



FIGURE 18. 3D reconstruction of enamel lesion in non-cavitated white spot enamel lesion from μ CT image analysis.

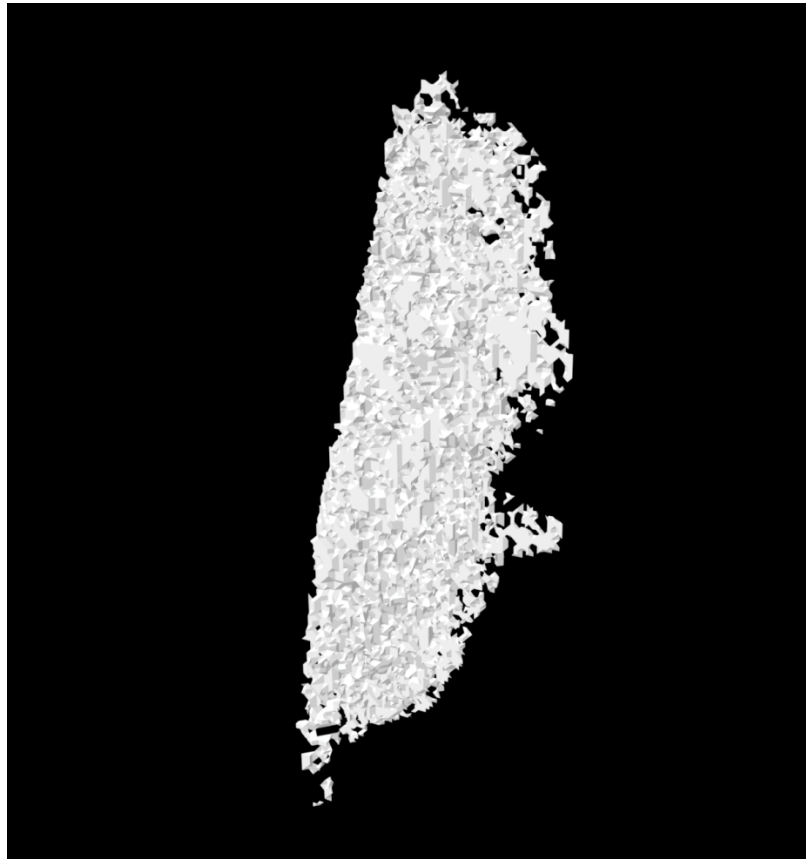


FIGURE 19. 3D reconstruction of dentine lesion in non-cavitated white spot enamel lesion from μ CT image analysis.

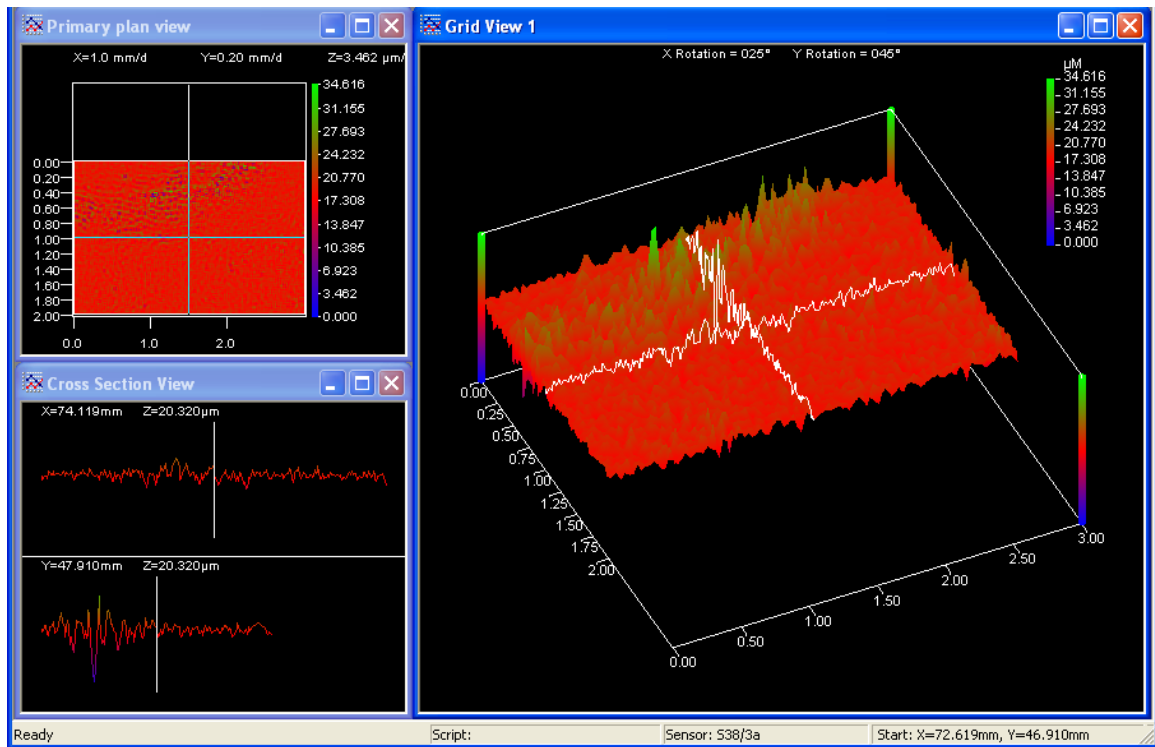


FIGURE 20. Non-contact profilometric analysis.

DISCUSSION

Caries lesions progress when the outcome of demineralization and remineralization processes over time is net mineral loss. Lesions that continue to demineralize are active, while those that display no evidence of further demineralization are rendered inactive.^{7,8} In several *in-vitro* studies of artificial non-cavitated early lesions, active lesions exhibited light reflection properties and surface layer thickness different from inactive lesions. Therefore, this *in-vitro* study sought to evaluate the difference between active and inactive natural non-cavitated early lesions in regard to surface layer thickness and the rate of fluorescence loss during dehydration while using QLF.

VISUAL/TACTILE EXAMINATION

Diagnosis of non-cavitated early lesions is challenging, even for the most experienced of clinicians. Correct assessment enables implementation of appropriate preventive and regenerative therapies, while incorrect assessment often leads to unnecessary restorative treatment, which increases the financial burden to the patient and condemns the tooth to a lifetime of subsequent re-restoration. Clinically, visual/tactile examination remains the “gold standard” in caries detection and diagnosis.^{31,43,46} However, tactile examination, in particular, suffers from poor predictive value.⁷⁹ Yet, nearly 25 years after the American Dental Association’s recommendation to avoid the use of sharp explorers,⁸⁰ many practitioners continue to rely heavily on the “catch” as proof of caries activity. The lack of acceptable alternative diagnostic methods has, undoubtedly, perpetuated this dilemma.

The dire need for a valid caries detection system gave rise to the ICDAS and later its modification ICDAS II + LAA (Lesion Activity Assessment) in 2005.³³ ICDAS has provided the worksheet that facilitates the appropriate management of dental caries in order to achieve healthy and effective long-term results. Therefore, in this current study we sought to validate the use of ICDAS II+ LAA with QLF fluorescence loss during dehydration by demonstrating a correlation between caries activity and surface layer thickness. Sample collection of non-cavitated white spot early lesions was carried out by two experienced examiners by consensus. One-hundred teeth with ICDAS 1 and 2 were selected for the study. Selection of teeth with lesions at the proposed dimension (2×5mm) with intact enamel surface has proved to be challenging. Lesions with such large dimensions on proximal surfaces are often cavitated, stained, or not homogenous. During teeth selection, the protocol indicated all teeth to be bleached in order to eliminate the variability of discoloration from the study. Teeth were bleached with 5.0-percent NaOCl to remove any stains that could cause bias in specimen selection. This protocol was used in a previous study for the purpose of acquiring optimal data.⁴² In the current study, teeth were bleached with 5.0-percent NaOCl for 30 minutes, rather than for 2 hours in the former study.⁴²

The negative effect of probing on demineralized intact enamel surface is well demonstrated through literature.⁸¹⁻⁸³ Probing can cause damage ranging from 100 µm to 2.0 mm on initial lesion of proximal surfaces.⁸² Such damage results in cavitation and lesion progression. This current study is the first to try to standardize the force applied by the explorer during the examination process. In an attempt to minimize the damage produced by dental explorers, the force applied while passively moving the explorer was

contained to a minimum and not exceeding 20 gm. Examiners measured the amount of force applied by passing the explorer on an electronic scale surface during training prior to initiation of tactile examination and after the examination of 50 teeth. Although our method was not used before, the main purpose of it was standardization of the minimum effective applied force able to distinguish surface texture.⁸¹⁻⁸³

SURFACE ROUGHNESS

$(Ra_{\text{lesion}} - Ra_{\text{sound}})/Ra_{\text{sound}}$ statistical analysis included the roughness of the sound area of the tooth in the outcome measurement, so that the natural variation in sound tooth surface roughness had less influence on the data when the roughness of the active and inactive caries portions of the tooth surfaces were compared. It directly explains how much larger (greater than 0) or smaller (less than 0) the lesion surface is compared with the sound surface. $(Ra_{\text{lesion}} - Ra_{\text{sound}})/Ra_{\text{sound}}$ was 0.26 for active lesions, which means that the active surfaces were 0.26 times higher in Ra, or 26 percent rougher, than the sound surfaces. Similarly, $(Ra_{\text{lesion}} - Ra_{\text{sound}})/Ra_{\text{sound}}$ was 0.22 for inactive lesions, which means that inactive surfaces were 0.22 times higher in Ra, or 22 percent rougher, than the sound surfaces. Active lesions showed a slightly higher Ra value than inactive lesions. These differences, however, (26% vs. 22%), were not statistically significant ($p = 0.839$).

The tactile criteria of caries activity assessment are well-recognized in the literature.^{12,31} As previously mentioned, active lesions generally present rough surfaces, while inactive lesions are smooth. This study evaluated the surface roughness (Ra) of natural white spot enamel lesions without destructive sectioning of teeth. It reported Ra values of 1.57 μm and 1.51 μm for active and inactive lesions respectively. Neuhaus et

al.¹⁴ reported a profilometric analysis of active and inactive lesions, in which corresponding surface roughness measurements values were 0.805 μm (0.567 μm to 1.051 μm) for active lesions and 0.411 μm (0.364 μm to 0.5 μm) for inactive lesions. Other studies evaluated surface roughness of artificial lesions introduced to flat surfaces of prepared enamel specimens.⁸⁴⁻⁸⁶ The literature on usage of non-contact profilometry to measure non-sectioned tooth structure is limited to enamel surface treatment⁸⁷ and adhesive clearance methods following orthodontic bracket debonding.⁸⁸⁻⁹⁰ An *in-vitro* study by Arnold et al.⁹¹ reported the median surface roughness (Ra) of untreated enamel lesions with ICDAS 1 and 2 on the proximal surfaces of premolars as 0.263 μm . This Ra value did not agree with our study, which was considerably higher.

SURFACE LAYER THICKNESS AND CARIES ACTIVITY

Our main hypothesis was to determine if visual/tactile active caries lesions present thinner surface layers than inactive lesions. Our results were unable to reject the null hypothesis, as active and inactive lesions demonstrated no significant difference in surface layer thickness (SLT). The surface layers of intact enamel lesions were manually traced on μCT images. Both 2D and 3D image analyses were implemented for two reasons: First, 2D image analysis, or the indirect image analysis method, is the traditional method of analysis.⁵⁴ It provided a single 2D average of each specimen, as well as a detailed point-by-point measurement of all specimens combined, which enabled us to measure the average, minimum, and maximum thickness of the surface. Second, the novel 3D approach (although considered more accurate)⁵⁴ provided only an overall measurement of the average surface layer thickness for each sample dataset. 3D analysis

was performed primarily to compare SLT of active and inactive lesions, while 2D was implemented to establish a range of SLT for active and inactive lesions.

The results of this current study agree with previous studies^{26,27,28} regarding the range of SLT. This study also agrees with Cochrane et al.,²⁶ as it found no significant difference in SLT between active and inactive enamel lesions. Shahmoradi and Swain²⁷ reported mean SLT of 56 μm ²⁷ for proximal lesions and mean SLT of 51 μm for WSL in another study,²⁸ while in the present study, the mean was 57 μm for active lesions and 52 μm for inactive lesions.

The present study raises a question about the wide variation among minimum and maximum surface layer thicknesses within both active and inactive lesions. SLTs were statistically similar, ranging from 25 μm to 92 μm within active lesions and 30 μm to 75 μm within inactive lesions (Table V). One explanation would be due to the large dimensions of the selected natural lesions; one lesion can contain a wide variation of mineralization levels.³⁰ A single natural lesion may contain areas of activity and inactivity, but overall can be assumed as active. The studies that found differences in SLT between active and inactive (or demineralized and remineralized) lesions mainly investigated artificial lesions.^{22,40,41,92} Artificial lesions tend to show a more uniform structure than natural lesions. Our finding is in agreement with Lee et al.,³⁰ who used PS-OCT to measure the integrated reflectivity of active and inactive enamel lesions in smooth surface and occlusal surface groups. They did not find a significant difference in the integrated reflectivity (ΔR) between active and inactive lesion groups of the smooth surface lesion group. However, a significant difference was found in the pits and fissures lesion group. Previous studies^{18,40,93} indicated that inactive lesions undergo a

remineralization process in which partially demineralized crystals and newly formed crystals increase in size within the lesion body, thus increasing the surface layer thickness. Since SL studies on natural lesions are limited, further investigations of the SL thickness in active and inactive natural non-cavitated enamel lesions on smooth surfaces and fissure surfaces are necessary. For instance, studies on the variation and distribution of SL thickness within active and inactive non-cavitated lesions are suggested. Investigations into whether active lesions contain a higher distribution of thin surface layer along the extension of the lesion than inactive ones may provide a better understanding of surface layer thickness and caries activity.

QLF DURING DEHYDRATION AND CARIES ACTIVITY

The current study showed a weak negative correlation between ΔQ_D at 15s dehydration and surface layer thickness 2D-Min and 2D-avg with PCC= -0.4 ($p = 0.026$) and -0.36 ($p = 0.049$), respectively. This finding suggests that the thinner the surface layer, the higher the rate of fluorescence loss, and therefore, the higher the lesion permeability. The study confirms that ΔQ_D can determine (or define) lesion activity in natural enamel lesions. ΔQ_D was significantly larger for active lesions at the first second of dehydration at 5.0-percent threshold ($p = 0.046$). This indicates that active lesions have the ability to lose water more rapidly than inactive lesions due to their higher surface porosity. Surface porosity is an indication of lesion activity. Active lesions show higher porosity than inactive lesions, which relates to mineral density.²³ Therefore, the more demineralized the surface, the more active the lesion. Ando et al.⁷⁸ previously demonstrated the ability of QLF during dehydration ΔQ per second (ΔQ_D) to determine lesion activity at the time of examination in artificial lesions. ΔQ_D has shown a rapid

increase in fluorescence loss rate in active lesions compared with remineralized lesions, thereby indicating its activity.⁷⁸ As caries lesions become inactive or arrested by mineral deposition through the outer enamel layer, the rate of fluid diffusion is reduced, rendering these lesions inactive. QLF during dehydration exploits the optical changes that occur as a result of water loss through porous caries lesions in the first few seconds of air-drying.⁷⁵

SURFACE POROSITY AND SURFACE ROUGHNESS

The study was not able to find a significant correlation between surface roughness Ra and surface layer thickness PCC = 0.00 for 2D-avg (p-value = 0.994). The correlation between ΔQ_D and surface roughness was not statistically significant. This finding suggests that roughness and porosity may be different entities and are not necessarily associated with each other.²³ A rough surface does not have to be porous as demonstrated in the inactive lesion group because they are two different phenomena.²³ Therefore, an inactive lesion may show a rough surface and no porosity.

QLF DURING DEHYDRATION AND SURFACE LAYER THICKNESS

The results of QLF during dehydration showed a weak negative correlation between ΔQ_D at 15-s dehydration and surface layer thickness per 2D-Min and 2D-avg. At 15 s of dehydration PCC = -0.40 (p = 0.026). QLF ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) and surface layer thickness 2D-avg were significantly correlated, with a weak negative association at QLF 5.0-percent threshold with 15 seconds dehydration (PCC = -0.36; p = 0.049). QLF ΔQ_D ($\% \times \text{mm}^2/\text{sec}$) was not significantly associated with surface layer thickness 3D (all p \geq 0.188), Therefore, the null hypothesis was rejected. In relation to caries lesion activity,

our second hypothesis sought to find an association between surface layer thickness and activity determined with QLF during dehydration.

In regard to associations of SLT and surface roughness, PCC was 0.12 for 3D SLT ($p = 0.529$), 0.07 for 2D ($p = 0.729$), 0.00 for 2D-avg ($p = 0.994$), -0.24 for 2D-min ($p = 0.209$), -0.05 for 2D-max ($p = 0.796$). We are unable to reject the third null hypothesis, which stated that visual/tactile active caries lesions present rougher surfaces than inactive lesions.

CARIES LESION SEVERITY ASSESSMENT

The present study showed that active caries lesions were significantly larger in volume than inactive caries lesions, whereas depth analysis showed a significant difference in only dentinal lesion depth between active and inactive lesions. Lesion depth in dentine was significantly larger for active lesions (Table VII; $p = 0.009$). Enamel and overall lesion volumes (mm^3) from μCT image analysis were significantly larger in active lesions than in inactive lesions ($p = 0.075$ for dentine lesion volume, 0.031 for enamel lesion volume, and 0.022 for overall lesion volume). One of the main advantages of using μCT image analysis is the ability to provide a 3D volumetric calculation of the scanned object. Unlike TMR and other histology gold standard methods such as polarized light-microscopy, μCT are able to provide volumetric information without damaging enamel samples. μCT has demonstrated its ability to evaluate bone and tooth mineral density with an accuracy of over 1.0 percent and a resolution between 5 μm and 30 μm .⁹⁴ It also allows rescanning of the sample in longitudinal studies. The disadvantages of such a method is mainly the scanning and analysis time for each sample and its high cost.

In the present study, we were able to conduct a volumetric analysis, as well as

measure caries lesion depth and compare the severity of active and inactive non-cavitated white spot lesions. In a study by Arnold et al.⁹⁵ volumetric assessment of natural proximal caries lesions indicated presence of more than one individual initial caries lesions at the proximal tooth surface. Natural caries lesions can be highly unpredictable in terms of mineral distribution; therefore, a three-dimensional volumetric analysis would provide more accurate information than a two-dimensional depth calculation.

Our study agrees with Kucuk et al.⁹⁶ confirming that volumetric analysis of microtomographic imaging is an effective method for evaluation of non-cavitated early enamel lesions. Kucuk et al.⁹⁶ utilized microtomographic imaging to measure volume, depth, surface area, and mineral density changes in enamel lesions through different remineralization procedures. Microtomographic imaging has shown great potentials as a gold standard method of measurement and assessment of progression and remineralization of early enamel lesions.

Our last aim was to determine the lesion severity by correlating lesion volume and maximum depth acquired by μ CT, and ΔQ value at 15 seconds from QLF during dehydration. We were able to find a moderate positive association between caries lesion volume and ΔQ at 15 seconds of dehydration. At 0.95 μ CT threshold and ΔQ at 15 seconds of dehydration, $PCC = 0.56$ ($p = 0.001$). However, ΔQ at 15 seconds of dehydration was not significantly associated with μ CT lesion depth. QLF has demonstrated its ability to monitor caries lesion progression and activity assessment.^{42,77,78} This current study sheds light on the ability of QLF during dehydration to assess the severity of caries lesions. Since natural lesion volume is a better indicator of lesion extension than two-dimensional depth measurement, it is safe to say that our result

confirms that ΔQ at 15 seconds of dehydration is able to assess caries severity. As the lesion increases in volume, the amount of fluorescence loss increases as well.

Within the limitations of this current study, our results in regard to QLF during dehydration confirm the ability of this method to aid in activity and severity assessment in addition to monitoring caries progression along with visual and tactile examinations. It can be a valuable asset for targeting actively progressing non-cavitated enamel lesions, which are normally most challenging to diagnose in clinical practice. μ CT imaging has shown to be a novel and promising conservative method to study and to assess natural non-cavitated, early enamel lesion surface layer and overall lesion volume *in vitro*. Although the present study was unable to establish a correlation between activity and surface layer thickness, it provided insight into the nature of the surface layer in natural enamel lesions, which can be a foundation for investigation in future studies.

SUMMARY AND CONCLUSIONS

Early detection and diagnosis of non-cavitated early lesions can be grueling, especially for inexperienced dental practitioners. Determination of caries activity is essential for targeted caries prevention therapy and management, which, if performed successfully, can drastically decrease the global burden. International Caries Detection and Assessment System and Lesion Activity Assessment (ICDAS + LAA) provide a valid method of caries detection and diagnosis, which ensures the management of dental caries while achieving healthy and effective long-term results. QLF during dehydration is a promising method of activity and severity assessment that may be used in conjunction with visual and tactile examination in a clinical setting.

Our main objective was to compare visual and tactile active and inactive natural non-cavitated early lesions in terms of surface roughness (Ra), μ CT lesion volume, μ CT lesion depth, μ CT surface layer thickness, QLF ΔQ , and QLF ΔQD . One hundred extracted teeth with ICDAS 1 and 2 proximal lesions were selected for V/T examination in this study. From these, 15 suspected active and 15 suspected inactive lesions were chosen by visual and tactile examinations by consensus of two experienced examiners after training.

Roughness measurements (Ra) were acquired using non-contact optical profilometry and $(Ra_{\text{lesion}} - Ra_{\text{sound}}) / Ra_{\text{sound}}$ was calculated. Thickness of SL, volume of lesions and their extent were determined by μ CT image analysis. A series of fluorescence images were acquired at baseline (hydrated), 1s, 5s, 10s and 15s by QLF. During the image acquisition, surfaces were dehydrated with continuous compressed air.

QLF-variables [average fluorescence loss (%), size (mm^2), ΔQ ($\% \times \text{mm}^2$)] and the change-in-QLF-variables per second (ΔQ_D) were determined. Two-sample t -tests were used for statistical comparisons, while scatterplots and Pearson correlation coefficients were used to find statistical associations.

The study reported Ra values of $1.57 \mu\text{m}$ and $1.51 \mu\text{m}$ for active and inactive lesions, respectively, which were larger values than those obtained from other studies. Active lesions presented a slightly rougher surface and higher Ra value than inactive lesion, but the difference was not significant ($p = 0.761$). Active lesions were 26 percent rougher than sound tooth surfaces while inactive lesions were 22 percent rougher.

Surface layer thickness ranged from $25 \mu\text{m}$ to $92 \mu\text{m}$ within active lesions and $30 \mu\text{m}$ to $75 \mu\text{m}$ within inactive lesions. The mean SLT was $57 \mu\text{m}$ for active lesions and $52 \mu\text{m}$ for inactive lesions. We were unable to reject the null hypothesis, as active and inactive lesions did not show a statistically significant difference in surface layer thickness.

The study confirmed that ΔQ_D can detect lesion activity in natural non-cavitated early lesions. ΔQ_D was significantly larger for active lesions at the first second of dehydration at 5.0-percent threshold ($p = 0.046$). The study also showed a weak negative correlation between ΔQ_D at 15 seconds of dehydration and surface layer thickness 2D-Min ($\text{PCC} = -0.4$; $p = 0.026$) and 2D-avg ($\text{PCC} = -0.36$; $p = 0.049$). This finding suggests that the thinner the surface layer, the higher the rate of fluorescence loss, and therefore, the higher the lesion permeability.

This *in-vitro* study showed that active caries lesions were significantly larger in volume than inactive caries lesions ($p = 0.022$ for overall lesion volume). In contrast,

depth analysis showed a significant difference only in dentinal lesion depth ($p = 0.009$) between active and inactive lesions. A moderate positive association between caries lesion volume and ΔQ at 15 seconds of dehydration was found. At 0.95 μCT threshold and ΔQ at 15 seconds of dehydration, $\text{PCC} = 0.56$ ($p = 0.001$). However, ΔQ at 15 seconds of dehydration was not significantly associated with μCT lesion depth.

In conclusion, under the conditions of this *in-vitro* study:

1. Active and inactive lesions did not show a significant difference in surface layer (SL) thickness.
2. Although QLF during dehydration (ΔQ_D) did not correlate well with SL thickness, ΔQ_D has the ability to determine lesion activity in natural enamel lesions; the thinner the surface layer, the higher the rate of fluorescence loss, and therefore, the higher the lesion permeability.
3. Active lesions presented a slightly rougher surface and higher Ra value than inactive lesions, but the difference was not statistically significant.
4. A moderate positive association between caries lesion volume and ΔQ at 15 seconds of dehydration suggests that the larger the lesion volume, the higher the fluorescence loss.

QLF during dehydration is a valid promising method for activity and severity assessment in conjunction with V/T examination. μCT is a novel gold standard method with the ability to provide three-dimensional information on SLT and overall lesion volume of non-cavitated early lesions in a non-destructive manner.

REFERENCES

1. Lagerweij MD, van Loveren C. Declining caries trends: are we satisfied? *Curr Oral Health Rep.* 2015;2(4):212-7.
2. Wathen WF. International implications of "oral health in America: a report of the Surgeon General". *Quintessence Int.* 2000;31(10):697.
3. Allukian M, Jr. The neglected epidemic and the surgeon general's report: a call to action for better oral health. *Am J Public Health.* 2008;98(9 Suppl):S82-85.
4. Frencken JE, Sharma P, Stenhouse L, Green D, Lavery D, Dietrich T. Global epidemiology of dental caries and severe periodontitis - a comprehensive review. *J Clin Periodontol.* 2017;44 Suppl 18:S94-S105.
5. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of untreated caries: a systematic review and metaregression. *J Dent Res.* 2015;94(5):650-8.
6. Baelum V. What is an appropriate caries diagnosis? *Acta Odontol Scand.* 2010;68(2):65-79.
7. Kugel G, Arsenault P, Papas A. Treatment modalities for caries management, including a new resin infiltration system. *Compend Contin Educ Dent.* 2009;30 Spec No 3:1-10; quiz 11-12.
8. Paula AB, Fernandes AR, Coelho AS, et al. Therapies for white spot lesions-a systematic review. *J Evid Based Dent Pract.* 2017;17(1):23-38.
9. Mount GJ. Defining, classifying, and placing incipient caries lesions in perspective. *Dent Clin North Am.* 2005;49(4):701-23, v.
10. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J.* 2006;51(4):284-9; quiz 347.
11. Pitts NB. Are we ready to move from operative to non-operative/preventive treatment of dental caries in clinical practice? *Caries Res.* 2004;38(3):294-304.
12. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. *Caries Res.* 1999;33(4):252-60.
13. Nyvad B, Machiulskiene V, Baelum V. Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. *J Dent Res.* 2003;82(2):117-22.
14. Neuhaus KW, Nyvad B, Lussi A, Jaruszewski L. Evaluation of perpendicular reflection intensity for assessment of caries lesion activity/inactivity. *Caries Res.* 2011;45(4):408-14.
15. Kuhnisch J, Berger S, Goddon I, Senkel H, Pitts N, Heinrich-Weltzien R. Occlusal caries detection in permanent molars according to WHO basic methods, ICDAS II and laser fluorescence measurements. *Community Dent Oral Epidemiol.* 2008;36(6):475-84.

16. Ismail AI, Tellez M, Pitts NB, et al. Caries management pathways preserve dental tissues and promote oral health. *Community Dent Oral Epidemiol*. 2013;41(1):e12-40.
17. Guedes RS, Piovesan C, Ardenghi TM, et al. Validation of Visual Caries Activity Assessment: A 2-yr Cohort Study. *J Dent Res*. 2014;93(7 Suppl):101S-107S.
18. Kidd EA. The histopathology of enamel caries in young and old permanent teeth. *Br Dent J* 1983;155(6):196-8.
19. Ekstrand KR, Ricketts DN, Kidd EA, Qvist V, Schou S. Detection, diagnosing, monitoring and logical treatment of occlusal caries in relation to lesion activity and severity: an in vivo examination with histological validation. *Caries Res* 1998;32(4):247-54.
20. ten Bosch JJ, Borsboom PC, ten Cate JM. A non-destructive optical method to study de- and remineralization of enamel. *J Dent Res* 1979;58(Spec Issue B):1027.
21. Arends J, ten Bosch JJ. Demineralization and remineralization evaluation techniques. *J Dent Res* 1992;71 Spec No:924-928.
22. Jones RS, Fried D. Remineralization of enamel caries can decrease optical reflectivity. *J Dent Res* 2006;85(9):804-8.
23. Jaruszewski L. Differentiation of enamel lesion activity by vertical reflection intensity - a methodical description. *Biomed Tech (Berl)* 2012;57(2):139-47.
24. Kielbassa AM, Gillmann L, Zantner C, Meyer-Lueckel H, Hellwig E, Schulte-Monting J. Profilometric and microradiographic studies on the effects of toothpaste and acidic gel abrasivity on sound and demineralized bovine dental enamel. *Caries Res* 2005;39(5):380-6.
25. Meyer-Lueckel H, Paris S, Kielbassa AM. Surface layer erosion of natural caries lesions with phosphoric and hydrochloric acid gels in preparation for resin infiltration. *Caries Res* 2007;41(3):223-30.
26. Cochrane NJ, Anderson P, Davis GR, Adams GG, Stacey MA, Reynolds EC. An X-ray microtomographic study of natural white-spot enamel lesions. *J Dent Res* 2012;91(2):185-91.
27. Shahmoradi M, Swain MV. Quantitative characterization and micro-CT mineral mapping of natural fissural enamel lesions. *J Dent* 2016;46:23-9.
28. Shahmoradi M, Swain MV. Micro-CT analysis of naturally arrested brown spot enamel lesions. *J Dent* 2017;56:105-11.
29. National Institutes of H. Diagnosis and management of dental caries throughout life. *NIH Consens Statement* 2001;18(1):1-23.
30. Lee RC, Staninec M, Le O, Fried D. Infrared methods for assessment of the activity of natural enamel caries lesions. *IEEE J Sel Top Quantum Electron* 2016;22(3).
31. Ekstrand KR, Zero DT, Martignon S, Pitts NB. Lesion activity assessment. *Monogr Oral Sci* 2009;21:63-90.
32. Braga MM, Ekstrand KR, Martignon S, Imparato JC, Ricketts DN, Mendes FM. Clinical performance of two visual scoring systems in detecting and assessing activity status of occlusal caries in primary teeth. *Caries Res* 2010;44(3):300-8.
33. Dikmen B. Icdas II criteria (international caries detection and assessment system). *J Istanbul Univ Fac Dent* 2015;49(3):63-72.

34. Watts A, Addy M. Tooth discolouration and staining: a review of the literature. *Br Dent J* 2001;190(6):309-16.
35. Dreizen S, Spies TD. A note of the production of a yellow-brown pigment in the organic matrices of noncarious human teeth by oral lactobacilli. *Oral Surg Oral Med Oral Pathol* 1950;3(5):686-91.
36. Kidd EA, Beighton D. Prediction of secondary caries around tooth-colored restorations: a clinical and microbiological study. *J Dent Res* 1996;75(12):1942-6.
37. Belkhir MS, Douki N. An new concept for removal of dental fluorosis stains. *J Endod* 1991;17(6):288-92.
38. Carey CM. Tooth whitening: what we now know. *J Evid Based Dent Pract* 2014;14 Suppl:70-6.
39. Penumatsa NV, Sharanesha RB. Bleaching of fluorosis stains using sodium hypochlorite. *J Pharm Bioallied Sci* 2015;7(Suppl 2):S766-768.
40. Jones RS, Darling CL, Featherstone JD, Fried D. Remineralization of in vitro dental caries assessed with polarization-sensitive optical coherence tomography. *J Biomed Opt* 2006;11(1):014016.
41. Jones RS, Darling CL, Featherstone JD, Fried D. Imaging artificial caries on the occlusal surfaces with polarization-sensitive optical coherence tomography. *Caries Res* 2006;40(2):81-9.
42. Ando M, Stookey GK, Zero DT. Ability of quantitative light-induced fluorescence (QLF) to assess the activity of white spot lesions during dehydration. *Am J Dent* 2006;19(1):15-8.
43. Ismail AI, Sohn W, Tellez M, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol* 2007;35(3):170-8.
44. Pitts N. "ICDAS"--an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. *Community Dent Health* 2004;21(3):193-8.
45. Ekstrand KR, Martignon S, Ricketts DJ, Qvist V. Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Oper Dent* 2007;32(3):225-35.
46. Jablonski-Momeni A, Stachniss V, Ricketts DN, Heinzl-Gutenbrunner M, Pieper K. Reproducibility and accuracy of the ICDAS-II for detection of occlusal caries in vitro. *Caries Res* 2008;42(2):79-87.
47. Diniz MB, Rodrigues JA, Hug I, Cordeiro Rde C, Lussi A. Reproducibility and accuracy of the ICDAS-II for occlusal caries detection. *Community Dent Oral Epidemiol* 2009;37(5):399-404.
48. Tikhonova SM, Feine JS, Pustavoitava NN, Allison PJ. Reproducibility and diagnostic outcomes of two visual-tactile criteria used by dentists to assess caries lesion activity: a cross-over study. *Caries Res* 2014;48(2):126-36.
49. Novaes TF, Matos R, Braga MM, Imparato JC, Raggio DP, Mendes FM. Performance of a pen-type laser fluorescence device and conventional methods in detecting approximal caries lesions in primary teeth--in vivo study. *Caries Res* 2009;43(1):36-42.
50. Hounsfield GN. Computerized transverse axial scanning (tomography). (Pt 1). Description of system. *Br J Radiol.* 1973;46(552):1016-22.

51. Kuhn JL, Goldstein SA, Feldkamp LA, Goulet RW, Jasion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. *J Orthop Res* 1990;8(6):833-42.
52. Guldberg RE, Ballock RT, Boyan BD, et al. Analyzing bone, blood vessels, and biomaterials with microcomputed tomography. *IEEE Eng Med Biol Mag* 2003;22(5):77-83.
53. Guldberg RE, Lin AS, Coleman R, Robertson G, Duvall C. Microcomputed tomography imaging of skeletal development and growth. *Birth Defects Res C Embryo Today* 2004;72(3):250-9.
54. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res* 1999;14(7):1167-74.
55. Huang TT, Jones AS, He LH, Darendeliler MA, Swain MV. Characterization of enamel white spot lesions using X-ray micro-tomography. *J Dent* 2007;35(9):737-43.
56. Kim I, Paik KS, Lee SP. Quantitative evaluation of the accuracy of micro-computed tomography in tooth measurement. *Clin Anat* 2007;20(1):27-34.
57. Hamba H, Nikaido T, Sadr A, Nakashima S, Tagami J. Enamel lesion parameter correlations between polychromatic micro-CT and TMR. *J Dent Res* 2012;91(6):586-91.
58. Soviero VM, Leal SC, Silva RC, Azevedo RB. Validity of MicroCT for in vitro detection of proximal carious lesions in primary molars. *J Dent* 2012;40(1):35-40.
59. Carvalho RN, Letieri ADS, Vieira TI, et al. Accuracy of visual and image-based ICDAS criteria compared with a micro-CT gold standard for caries detection on occlusal surfaces. *Braz Oral Res*. 2018;32:e60.
60. Angmar-Mansson B, ten Bosch JJ. Quantitative light-induced fluorescence (QLF): a method for assessment of incipient caries lesions. *Dentomaxillofac Radiol* 2001;30(6):298-307.
61. Hafstrom-Bjorkman U, Sundstrom F, ten Bosch JJ. Fluorescence in dissolved fractions of human enamel. *Acta Odontol Scand* 1991;49(3):133-8.
62. Ogaard B, Ten Bosch JJ. Regression of white spot enamel lesions. A new optical method for quantitative longitudinal evaluation in vivo. *Am J Orthod Dentofacial Orthop* 1994;106(3):238-42.
63. Emami Z, al-Khateeb S, de Josselin de Jong E, Sundstrom F, Trollas K, Angmar-Mansson B. Mineral loss in incipient caries lesions quantified with laser fluorescence and longitudinal microradiography. A methodologic study. *Acta Odontol Scand* 1996;54(1):8-13.
64. Ando M, Hall AF, Eckert GJ, Schemehorn BR, Analoui M, Stookey GK. Relative ability of laser fluorescence techniques to quantitate early mineral loss in vitro. *Caries Res* 1997;31(2):125-31.
65. al-Khateeb S, Oliveby A, de Josselin de Jong E, Angmar-Mansson B. Laser fluorescence quantification of remineralisation in situ of incipient enamel lesions: influence of fluoride supplements. *Caries Res* 1997;31(2):132-40.

66. van der Veen MH, de Josselin de Jong E. Application of quantitative light-induced fluorescence for assessing early caries lesions. *Monogr Oral Sci* 2000;17:144-62.
67. Tranaeus S, Al-Khateeb S, Bjorkman S, Twetman S, Angmar-Mansson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. *Eur J Oral Sci* 2001;109(2):71-5.
68. Shi XQ, Tranaeus S, Angmar-Mansson B. Comparison of QLF and DIAGNOdent for quantification of smooth surface caries. *Caries Res* 2001;35(1):21-6.
69. Ando M, van Der Veen MH, Schemehorn BR, Stookey GK. Comparative study to quantify demineralized enamel in deciduous and permanent teeth using laser- and light-induced fluorescence techniques. *Caries Res* 2001;35(6):464-70.
70. Pretty IA. Caries detection and diagnosis: novel technologies. *J Dent* 2006;34(10):727-39.
71. Stookey GK. Optical methods--quantitative light fluorescence. *J Dent Res* 2004;83 Spec No C:C84-88.
72. Ferreira Zandona A, Santiago E, Eckert G, Fontana M, Ando M, Zero DT. Use of ICDAS combined with quantitative light-induced fluorescence as a caries detection method. *Caries Res* 2010;44(3):317-22.
73. Ferreira Zandona A, Ando M, Gomez GF, et al. Longitudinal analyses of early lesions by fluorescence: an observational study. *J Dent Res* 2013;92(7 Suppl):84S-89S.
74. Felix Gomez G, Eckert GJ, Ferreira Zandona A. Orange/Red Fluorescence of active caries by retrospective quantitative light-induced fluorescence image analysis. *Caries Res*. 2016;50(3):295-302.
75. Mujat C, van der Veen MH, Ruben JL, Dogariu A, ten Bosch JJ. The influence of drying on quantitative laser fluorescence and optical pathlengths in incipient natural caries lesions. *Caries Res* 2004;38(5):484-92.
76. Al-Khateeb S, Exterkate RA, de Josselin de Jong E, Angmar-Mansson B, ten Cate JM. Light-induced fluorescence studies on dehydration of incipient enamel lesions. *Caries Res* 2002;36(1):25-30.
77. Ando M, Ferreira-Zandona AG, Eckert GJ, Zero DT, Stookey GK. Pilot clinical study to assess caries lesion activity using quantitative light-induced fluorescence during dehydration. *J Biomed Opt* 2017;22(3):35005.
78. Ando M, Fontana M, Eckert GJ, Arthur RA, Zhang H, Zero DT. Objective and quantitative assessment of caries lesion activity. *J Dent* 2018;78:76-82.
79. Ismail AI. Visual and visuo-tactile detection of dental caries. *J Dent Res* 2004;83 Spec No C:C56-66.
80. Caries diagnosis and risk assessment. A review of preventive strategies and management. *J Am Dent Assoc* 1995;126 Suppl:1S-24S.
81. Ekstrand K, Qvist V, Thylstrup A. Light microscope study of the effect of probing in occlusal surfaces. *Caries Res* 1987;21(4):368-74.
82. Kuhnisch J, Dietz W, Stosser L, Hickel R, Heinrich-Weltzien R. Effects of dental probing on occlusal surfaces--a scanning electron microscopy evaluation. *Caries Res* 2007;41(1):43-8.

83. Mattos-Silveira J, Oliveira MM, Matos R, Moura-Netto C, Mendes FM, Braga MM. Do the ball-ended probe cause less damage than sharp explorers?-An ultrastructural analysis. *BMC Oral Health* 2016;16:39.
84. Groenhuis RA, Jongebloed WL, ten Bosch JJ. Surface roughness of acid-etched and demineralized bovine enamel measured by a laser speckle method. *Caries Res* 1980;14(5):333-40.
85. Zhang XZ, Anderson P, Dowker SE, Elliott JC. Optical profilometric study of changes in surface roughness of enamel during in vitro demineralization. *Caries Res* 2000;34(2):164-74.
86. Ando M, Eckert GJ, Zero DT. Preliminary study to establish a relationship between tactile sensation and surface roughness. *Caries Res* 2010;44(1):24-8.
87. Ersahan S, Alakus Sabuncuoglu F. Effect of surface treatment on enamel surface roughness. *J Istanbul Univ Fac Dent* 2016;50(1):1-8.
88. Sigilliao LC, Marquezan M, Elias CN, Ruellas AC, Sant'Anna EF. Efficiency of different protocols for enamel clean-up after bracket debonding: an in vitro study. *Dental Press J Orthod* 2015;20(5):78-85.
89. Fan XC, Chen L, Huang XF. Effects of various debonding and adhesive clearance methods on enamel surface: an in vitro study. *BMC Oral Health* 2017;17(1):58.
90. Mhatre AC, Tandur AP, Reddy SS, Karunakara BC, Baswaraj H. Enamel surface evaluation after removal of orthodontic composite remnants by intraoral sandblasting technique and carbide bur technique: a three-dimensional surface profilometry and scanning electron microscopic study. *J Int Oral Health* 2015;7(Suppl 2):34-39.
91. Arnold WH, Meyer AK, Naumova EA. Surface roughness of initial enamel caries lesions in human teeth after resin infiltration. *Open Dent J* 2016;10:505-15.
92. Lee RC, Kang H, Darling CL, Fried D. Automated assessment of the remineralization of artificial enamel lesions with polarization-sensitive optical coherence tomography. *Biomed Opt Express* 2014;5(9):2950-62.
93. Areida SK, Reinhardt DP, Muller PK, et al. Properties of the collagen type XVII ectodomain. Evidence for n- to c-terminal triple helix folding. *J Biol Chem* 2001;276(2):1594-1601.
94. Wong FS, Anderson P, Fan H, Davis GR. X-ray microtomographic study of mineral concentration distribution in deciduous enamel. *Arch Oral Biol* 2004;49(11):937-44.
95. Arnold WH, Gaengler P, Saeuberlich E. Distribution and volumetric assessment of initial approximal caries lesions in human premolars and permanent molars using computer-aided three-dimensional reconstruction. *Arch Oral Biol* 2000;45(12):1065-71.
96. Kucuk EB, Malkoc S, Demir A. Microcomputed tomography evaluation of white spot lesion remineralization with various procedures. *Am J Orthod Dentofacial Orthop* 2016;150(3):483-90.

ABSTRACT

ABILITY OF CARIES DETECTION METHODS
TO DETERMINE CARIES LESION
ACTIVITY

by

Fatma Aldawood

Indiana University School of Dentistry
Indianapolis, Indiana

Background: Non-cavitated caries lesions form due to acid diffusion and demineralization of enamel subsurface with an intact surface layer (SL). Caries lesions progress when the outcome of demineralization and remineralization processes over time is net mineral loss. Lesions that continue to demineralize are called active, while those that display no evidence of further demineralization are called inactive. Micro-computed-tomography (μ CT) analysis provides objective non-destructive measurements of the thickness of the surface layer (SL) and severity of caries lesions.

Aims: 1) To investigate if visual/tactile suspected active non-cavitated early white spot lesions present a thinner surface layer than inactive ones; 2) To investigate if there is an association between the thickness of the surface layer (SLT) and caries activity, as determined by QLF during dehydration (ΔQ_D); 3) To determine lesion severity by comparing lesion volume and maximum depth correlation with ΔQ value at 15 s from QLF during dehydration.

Materials and Methods: Thirty extracted human premolars exhibiting non-cavitated approximal white spot early lesions stored in 0.1.-percent thymol/4C° and treated with 5.0-percent NaOCl/30 min were included in the study. Fifteen active and 15 inactive lesions were determined by visual/tactile examinations by consensus of two experienced examiners. Roughness measurements (Ra) were acquired using non-contact optical profilometry. Two-dimensional minimum (2D-min), maximum (2D-max), average (2D-avg) SL and three-dimensional (3D) analyses, volume and depth of lesions were determined from μ CT image analysis. A series of fluorescence images were acquired at baseline (hydrated), at 1 s, at 5 s, at 10 s and at 15 s by QLF. During image acquisition, surfaces were dehydrated with continuous-compressed-air. ΔQ and $\Delta Q/s$ (ΔQ_D) were calculated. Data were analyzed using two-sample t-tests and Pearson correlation coefficients ($p < 0.05$).

Results: Surface roughness of active and inactive lesions was not significantly different ($p > 0.08$). Overall lesion volume and depth in dentin were significantly larger in active lesions ($p = 0.022$, $p = 0.009$). SL thickness of active and inactive lesions was not significantly different (2D = 0.121, 3D = 0.080, 2D-avg = 0.446, 2D-min = 0.197, 2D-max = 0.122). ΔQ_D at 1s was significantly larger for active lesions ($p = 0.046$). ΔQ at

15 s of dehydration had a moderate positive association with lesion volume ($r = 0.56$).

ΔQ_D had a weak negative association with SL thickness (2D-avg) and (2D-min).

Conclusions: 1) Active and inactive non-cavitated lesions show no difference in SL thickness; 2) QLF during dehydration (ΔQ_D) does not correlate well with SL thickness; 3) ΔQ at 15 s of dehydration correlates moderately well with lesion volume and is consistent with caries activity assessed by visual/tactile examination.

CURRICULUM VITAE

Fatma A. Aldawood

EDUCATION

- | | |
|------|---|
| 2010 | Bachelor of Dental Medicine and Surgery (BDS), King Abdulaziz University, Faculty of Dentistry, Jeddah, Saudi Arabia. |
| 2019 | MSD, Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry. |

PROFESSIONAL

- | | |
|--------------|--|
| 2012 to 2015 | General Dentist in King Fahad Armed Forces Hospital – Jeddah, Saudi Arabia |
|--------------|--|